WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Monday, July 09, 2007

Hide?	Set Name	Query	Hit Count
		DB=PGPB; THES=ASSIGNEE; PLUR=YES; OP=ADJ	
☐	L9	L8 and (gly-266 or g266 or g-266)	3
☐	L8	L7 and 266	79
☐	L7	L5 and (variant or mut$8)	532
☐	L6	L1 and (variant or mut$8)	0
☐	L5	(lipase or phospholipase or lipolitic enzyme) and lanuginosa	621
		DB=USPT,USOC,EPAB,JPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ	
☐	L4	L3 and (gly-266 or g266 or g-266)	0
☐	L3	L2 and 266	70
☐	L2	L1 and (variant or mut$8)	659
☐	L1	(lipase or phospholipase or lipolitic enzyme) and lanuginosa	988

END OF SEARCH HISTORY

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FILE 'HOME' ENTERED AT 17:13:15 ON 09 JUL 2007

=> file .nash

=> s lanuginosa and (lipase or phospholipase or lipolytic enzyme) and (variant or mut?)
L1          24 FILE MEDLINE
L2          77 FILE CAPLUS
L3          61 FILE SCISEARCH
L4          13 FILE LIFESCI
L5          28 FILE BIOSIS
L6          31 FILE EMBASE

TOTAL FOR ALL FILES
L7         234 LANUGINOSA AND (LIPASE OR PHOSPHOLIPASE OR LIPOLYTIC ENZYME)
               AND (VARIANT OR MUT?)

=> s l7 and 266
L8           0 FILE MEDLINE
L9           0 FILE CAPLUS
L10          0 FILE SCISEARCH
L11          0 FILE LIFESCI
L12          0 FILE BIOSIS
L13          0 FILE EMBASE

TOTAL FOR ALL FILES
L14          0 L7 AND 266

=> s l7 and 263
L15          0 FILE MEDLINE
L16          0 FILE CAPLUS
L17          0 FILE SCISEARCH
L18          0 FILE LIFESCI
L19          0 FILE BIOSIS
L20          0 FILE EMBASE

TOTAL FOR ALL FILES
L21          0 L7 AND 263

=> d l7 1

L7   ANSWER 1 OF 234     MEDLINE on STN
AN   2006408429     MEDLINE  Full-text
DN   PubMed ID: 16784752
TI   Role of solvation barriers in protein kinetic stability.
AU   Rodriguez-Larrea David; Minning Stefan; Borchert Torben V; Sanchez-Ruiz
     Jose M
CS   Departamento de Quimica Fisica, Facultad de Ciencias, 18071-Granada,
     Spain.
SO   Journal of molecular biology, (2006 Jul 14) Vol. 360, No. 3, pp. 715-24.
     Electronic Publication: 2006-05-19.
     Journal code: 2985088R. ISSN: 0022-2836.
CY   England: United Kingdom
DT   Journal; Article; (JOURNAL ARTICLE)
     (RESEARCH SUPPORT, NON-U.S. GOV'T)
LA   English
FS   Priority Journals
EM   200608
ED   Entered STN: 11 Jul 2006
     Last Updated on STN: 17 Aug 2006
     Entered Medline: 16 Aug 2006


=> s l7 not 2000-2007/py
L22          8 FILE MEDLINE
L23         25 FILE CAPLUS
L24         23 FILE SCISEARCH
L25          8 FILE LIFESCI
L26         10 FILE BIOSIS
L27         12 FILE EMBASE

TOTAL FOR ALL FILES
L28         86 L7 NOT 2000-2007/PY
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=> dup rem l28
PROCESSING COMPLETED FOR L28
L29 37 DUP REM L28 (49 DUPLICATES REMOVED)

=> d ibib abs 1-37

L29 ANSWER 1 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1999:231214 CAPLUS Full-text
DOCUMENT NUMBER: 130:264043
TITLE: Lipase variants and their use in detergent compositions
INVENTOR(S): Svendsen, Allan; Patkar, Shamkant Anant; Gormsen, Erik; Clausen, Ib Groth; Okkels, Jens Sigurd; Thellersen, Marianne
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: U.S., 50 pp., Cont.-in-part of U.S. Ser. No. 434,904, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
US 5892013	A	19990406	US 1995-488271		19950905
CA 2092615	A1	19920314	CA 1991-2092615		19910913
PRIORITY APPLN. INFO.:			DK 1990-2194	A	19900913
			DK 1990-2195	A	19900913
			DK 1990-2196	A	19900913
			US 1993-977429	B1	19930222
			DK 1993-466	A	19930423
			DK 1994-217	A	19940222
			US 1995-434904	B2	19950501

AB The present invention relates to lipase variants which exhibit improved properties, detergent compns. comprising said lipase variants, DNA constructs coding for said lipase variants, and methods of making said lipase variants. The variants contain one or more deletions or substitutions of an amino acid residue in the lipid contact zone, or substitutions of two amino acid residues of the surface loop structure with cysteine which are positioned to form a disulfide bond. Numerous Humicola lanuginosa lipase variants were prepared with recombinant Aspergillus oryzae. Variants R209A+E210A, E56Q, D96L, D96K, D96W, and D96W+E210N had considerably better wash performance than the wild-type lipase. A number of the variants had higher specific activity and/or improved resistance to proteolytic degradation and almost all had improved resistance to alkaline conditions.
REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 2 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1999:111576 CAPLUS Full-text
DOCUMENT NUMBER: 130:164901
TITLE: Lipase variants and their use in detergent compositions
INVENTOR(S): Svendsen, Allan; Patkar, Shamkant Anant; Gormsen, Erik; Okkels, Jens Sigurd; Thellersen, Marianne
PATENT ASSIGNEE(S): Novo Nordisk A/S, Norway
SOURCE: U.S., 51 pp., Cont.-in-part of U.S. Ser. No. 434,904, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
US 5869438	A	19990209	US 1995-479275		19950607
CA 2092615	A1	19920314	CA 1991-2092615		19910913
PRIORITY APPLN. INFO.:			DK 1990-2194	A	19900913
			DK 1990-2195	A	19900913
			DK 1990-2196	A	19900913
			US 1991-977429	B1	19910913
			DK 1993-466	A	19930423
			DK 1994-217	A	19940222
			US 1995-434904	B2	19950501

AB The present invention relates to lipase variants which exhibit improved properties and to detergent additives and detergent compns. comprising said lipase variants. The variants contain one or more deletions or substitutions of an amino acid residue in the lipid contact zone, or substitutions of two amino acid residues of the surface loop structure with cysteine which are positioned to form a disulfide bond. Numerous Humicola lanuginosa lipase variants were prepared with recombinant Aspergillus oryzae. Variants R209A+E210A, E56Q, D96L, D96K, D96W, and D96W+E210N had considerably better wash performance than the wild-type lipase. A number of the variants had higher specific activity and/or improved resistance to proteolytic degradation and almost all had improved resistance to alkaline conditions.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 3 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:785630 SCISEARCH Full-text
THE GENUINE ARTICLE: 246MN
TITLE: Computational analysis of chain flexibility and fluctuations in Rhizomucor miehei lipase
AUTHOR: Peters G H; Bywater R P (Reprint)
CORPORATE SOURCE: Novo Nordisk AS, Biostruct Grp, DK-2760 Malov, Denmark (Reprint); Tech Univ Denmark, Dept Chem, DK-2800 Lyngby, Denmark
COUNTRY OF AUTHOR: Denmark
SOURCE: PROTEIN ENGINEERING, (SEP 1999) Vol. 12, No. 9, pp. 747-754.
ISSN: 0269-2139.
PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 77
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have performed molecular dynamics simulation of Rhizomucor miehei lipase (Rml) with explicit water molecules present. The simulation was carried out in periodic boundary conditions and conducted for 1.2 ns in order to determine the concerted protein dynamics and to examine how well the essential motions are preserved along the trajectory. Protein motions are extracted by means of the essential dynamics analysis method for different lengths of the trajectory. Motions described by eigenvector 1 converge after approximately 200 ps and only small changes are observed with increasing simulation time. Protein dynamics along eigenvectors with larger indices, however, change with simulation time and generally, with increasing eigenvector index, longer simulation times are required for observing similar protein motions (along a particular eigenvector). Several regions in the protein show relatively large fluctuations and in particular motions in the active site lid and the segments Thr57-Asn63 and the active site hinge region Pro101-Gly104 are seen along several eigenvectors. These motions are generally associated with glycine residues, while no direct correlations are observed between these fluctuations and the positioning of prolines in the protein structure. The partial opening/closing of the lid is an example of induced fit mechanisms seen in other enzymes and could be a general mechanism for the activation of Rml.

L29 ANSWER 4 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1999:50858 SCISEARCH Full-text
THE GENUINE ARTICLE: 155QB
TITLE: Stereoselectivity of Mucorales lipases toward triradylglycerols - A simple solution to a complex problem
AUTHOR: Scheib H; Pleiss J; Kovac A; Paltauf F; Schmid R D (Reprint)
CORPORATE SOURCE: Univ Stuttgart, Inst Tech Biochem, Allmandring 31, D-70569 Stuttgart, Germany (Reprint); Univ Stuttgart, Inst Tech Biochem, D-70569 Stuttgart, Germany; Graz Tech Univ, Dept Biochem & Food Chem, A-8010 Graz, Austria
COUNTRY OF AUTHOR: Germany; Austria
SOURCE: PROTEIN SCIENCE, (JAN 1999) Vol. 8, No. 1, pp. 215-221.
ISSN: 0961-8368.
PUBLISHER: COLD SPRING HARBOR LAB PRESS, 1 BUNGTOWN RD, PLAINVIEW, NY 11724 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 21
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The lipases from Rhizopus and Rhizomucor are members of the family of Mucorales lipases. Although they display high sequence homology, their stereoselectivity toward triradylgrycerols (sn-2 substituted triacylglycerols) varies. Four different triradylglycerols were investigated, which were classified into two groups: flexible substrates with rotatable O'-C1' ether or ester bonds adjacent to C2 of glycerol and rigid substrates with a rigid N'-C1' amide bond or a phenyl ring in sn-2. Although Rhizopus lipase shows opposite stereopreference for flexible and rigid substrates (hydrolysis in sn-1 and sn-3, respectively), Rhizomucor lipase hydrolyzes both groups of triradylglycerols preferably in sn-1. To explain these experimental observations, computer-aided molecular modeling was applied to study the molecular basis of stereoselectivity. A generalized model for both lipases of the Mucorales family highlights the residues mediating stereoselectivity: (1) L258, the C-terminal neighbor of the catalytic histidine, and (2) G266, which is located in a loop contacting the glycerol backbone of a bound substrate. Interactions with triradylglycerol substrates are dominated by van der Waals contacts. Stereoselectivity can be predicted by analyzing the value of a single substrate torsion angle that discriminates between sn-1 and sn-3 stereopreference for all substrates and lipases investigated here. This simple model can be easily applied in enzyme and substrate engineering to predict Mucorales lipase variants and synthetic substrates with desired stereoselectivity.

L29 ANSWER 5 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1999:287580 CAPLUS Full-text
DOCUMENT NUMBER: 131:84811
TITLE: Steady state fluorescence studies on the interaction of Humicola lanuginosa lipase with lipid vesicles
AUTHOR(S): Cajal, Y.; Prat, J.; Svendsen, A.; De Bolos, J.; Alsina, M. A.
CORPORATE SOURCE: Department of Physical Chemistry, School of Pharmacy, University of Barcelona, Barcelona, 08028, Spain
SOURCE: Biomedical Chromatography (1999), 13(2), 157-158
CODEN: BICHE2; ISSN: 0269-3879
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In this work, the conformational changes that take place in Humicola lanuginosa lipase (HLL) upon binding to vesicles of 1-palmitoyl-2-oleoylglycero-sn-phosphoglycerol (POPG), and also in the presence of tributyrin (TB) as substrate, were studied by fluorescence techniques. The catalytic activity of the enzyme under the same conditions as used for fluorescence was measured spectroscopically by UV using p-nitrophenylbutyrate as substrate. An inactive mutant, with active site Ser-146 mutated to Ala, was used in the fluorescence expts. to avoid interference from product formation.
REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 6 OF 37 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1998400942 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 9730809
TITLE: Active serine involved in the stabilization of the active site loop in the Humicola lanuginosa lipase.
AUTHOR: Peters G H; Svendsen A; Langberg H; Vind J; Patkar S A; Toxvaerd S; Kinnunen P K
CORPORATE SOURCE: Chemistry Department III, H.C. Orsted Institutet, University of Copenhagen, Denmark.. ghp@st.ki.ku.dk
SOURCE: Biochemistry, (1998 Sep 8) Vol. 37, No. 36, pp. 12375-83.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 23 Feb 1999
Last Updated on STN: 23 Feb 1999
Entered Medline: 5 Feb 1999
AB We have investigated the binding properties of and dynamics in Humicola lanuginosa lipase (Hll) and the inactive mutant S146A (active Ser146 substituted with Ala) using fluorescence spectroscopy and molecular dynamics simulations, respectively. Hll and S146A show significantly different binding behavior for phosphatidylcholine (PC) and phosphatidylglycerol (PG) liposomes. Generally, higher binding affinity is observed for Hll than the S146A mutant. Furthermore, depending on the matrix, the addition of the transition state analogue benzene boronic acid increases the binding affinity of S146A, whereas only small changes are observed for Hll suggesting that the active site

lid in the latter opens more easily and hence more lipase molecules are bound to the liposomes. These observations are in agreement with molecular dynamics simulations and subsequent essential dynamics analyses. The results reveal that the hinges of the active site lid are more flexible in the wild-type Hll than in S146A. In contrast, larger fluctuations are observed in the middle region of the active site loop in S146A than in Hll. These findings reveal that the single mutation (S146A) of the active site serine leads to substantial conformational alterations in the H. lanuginosa lipase and different binding affinities.

L29 ANSWER 7 OF 37 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1998244839 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 9578545
TITLE: Interfacial activation of triglyceride lipase from Thermomyces (Humicola) lanuginosa: kinetic parameters and a basis for control of the lid.
AUTHOR: Berg O G; Cajal Y; Butterfoss G L; Grey R L; Alsina M A; Yu B Z; Jain M K
CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of Delaware, Newark 19716, USA.
CONTRACT NUMBER: GM29703 (NIGMS)
SOURCE: Biochemistry, (1998 May 12) Vol. 37, No. 19, pp. 6615-27. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: (COMPARATIVE STUDY) Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T) (RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 11 Jun 1998
Last Updated on STN: 29 Jan 1999
Entered Medline: 4 Jun 1998

AB A strategy is developed to analyze steady-state kinetics for the hydrolysis of a soluble substrate partitioned into the interface by an enzyme at the interface. The feasibility of this approach to obtain interfacial primary kinetic and equilibrium parameters is demonstrated for a triglyceride lipase. Analysis for phospholipase A2 catalyzed hydrolysis of rapidly exchanging micellar (Berg et al. (1997) Biochemistry 36, 14512-14530) and nonexchangeable vesicular (Berg et al., (1991) Biochemistry 30, 7283-7291) phospholipids is extended to include the case of a substrate that does not form the interface. The triglyceride lipase (tlTGL) from Thermomyces (formerly Humicola) lanuginosa hydrolyzes p-nitrophenylbutyrate or tributyrin partitioned in the interface of 1-palmitoyl-2-oleoyl-sn-glycero-3- phosphoglycerol (POPG) vesicles at a rate that is more than 100-fold higher than that for the monodispersed substrate or for the substrate partitioned into zwitterionic vesicles. Catalysis and activation is not seen with the S146A mutant without the catalytic serine-146; however, it binds to the POPG interface with the same affinity as the WT. Thus POPG acts as a diluent surface to which the lipase binds in an active, or "open", form for the catalytic turnover; however, the diluent molecules have poor affinity for the active site. Analysis of the substrate and the diluent concentration dependence of the rate of hydrolysis provides a basis for the determination of the primary interfacial catalytic parameters. As a competitive substrate, tributyrin provided a check for the apparent affinity parameters. Nonidealities from the fractional difference in the molecular areas in interfaces are expressed as the area correction factor and can be interpreted as a first-order approximation for the interfacial activity coefficient. The basis for the interfacial activation of tlTGL on anionic interface is attributed to cationic R81, R84, and K98 in the "hinge" around the 86-93 "lid" segment of tlTGL.

L29 ANSWER 8 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 1998:711158 CAPLUS Full-text
DOCUMENT NUMBER: 130:92064
TITLE: Spectroscopic studies of the interfacial binding of Humicola lanuginosa lipase
AUTHOR(S): Cajal, Yolanda; Prat, Josefina; De Bolos, Jordi; Asuncion Alsina, M.; Svendsen, Allan
CORPORATE SOURCE: School of Pharmacy, Department of Physical Chemistry, University of Barcelona, Barcelona, 08028, Spain
SOURCE: Analyst (Cambridge, United Kingdom) (1998), 123(11), 2229-2233
CODEN: ANALAO; ISSN: 0003-2654
PUBLISHER: Royal Society of Chemistry
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The interaction of Humicola lanuginosa lipase (HLL) with small unilamellar vesicles of 1-palmitoyl-2-oleoylglycero-sn-3- phosphoglycerol (POPG) and in the presence of tributyrin (TB) as a substrate was studied by the use of steady-state fluorescence techniques. An inactive mutant with

the serine from the catalytic triad changed by alanine (S146A) was used in expts. with TB to avoid interferences from product formation. HLL binds to POPG vesicles in an active or open form for the catalytic turnover, therefore POPG provides a suitable system for studying the conformational changes involving the movement of the loop of amino acids that covers the active site of the enzyme in solution Tryptophan (Trp) fluorescence shows that HLL binding to POPG occurs with a change in the environment of Trp residue(s) and that there is only one type of bound form, even in the presence of TB. Accessibility to aqueous quenchers indicates shielding of Trp in the membrane. Fluorescence anisotropy of the enzyme increases on binding to the vesicles, indicating restricted rotational freedom for the Trp due to penetration in the bilayer. Resonance energy transfer expts. using an interfacial membrane probe, 1-[4-(trimethylammonio)phenyl]-6-phenylhexa- 1,3,5-triene p-toluenesulfonate (TMA-DPH), and an internal membrane probe, 1,6-diphenylhexa-1,3,5-triene (DPH), indicate that HLL does not penetrate very deeply in the hydrophobic core of the membrane, but preferentially stays close to the lipid interface. Addition of substrate (TB) does not result in any addnl. changes in the spectroscopic properties of HLL. It is suggested that the observed changes are due to the 'opening of the lid' on binding to POPG vesicles, leaving the active site accessible for the substrate to bind.

REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 9 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:229525 SCISEARCH Full-text
THE GENUINE ARTICLE: ZC005
TITLE: A proposed architecture for lecithin cholesterol acyl transferase (LCAT): Identification of the catalytic triad and molecular modeling
AUTHOR: Peelman F; Vinaimont N; Verhee A; Vanloo B; Verschelde J L; Labeur C; Seguret-Mace S; Duverger N; Hutchinson G; Vandekerckhove J; Tavernier J; Rosseneu M (Reprint)
CORPORATE SOURCE: Univ Ghent, Dept Biochem, Lab Lipoprot Chem, Hosp Str 13, B-9000 Ghent, Belgium (Reprint); Univ Ghent, Dept Biochem, Lab Lipoprot Chem, B-9000 Ghent, Belgium; State Univ Ghent VIB, Fac Med, Dept Biochem, B-9000 Ghent, Belgium; Rhone Poulenc Rorer, GENCELL, Cardiovasc Dept, Vitry Sur Seine, France; Univ Coll London, Dept Biochem, London, England maryvonne.rosseneu@rug.ac.be
COUNTRY OF AUTHOR: Belgium; France; England
SOURCE: PROTEIN SCIENCE, (MAR 1998) Vol. 7, No. 3, pp. 587-599. ISSN: 0961-8368.
PUBLISHER: COLD SPRING HARBOR LAB PRESS, PUBLICATIONS DEPT, 500 SUNNYSIDE BLVD, WOODBURY, NY 11797-2924 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 52
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 15 Mar 2007

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The enzyme cholesterol lecithin acyl transferase (LCAT) shares the Ser/Asp-Glu/His triad with lipases, esterases and proteases, but the low level of sequence homology between LCAT and these enzymes did not allow for the LCAT fold to be identified yet. We, therefore, relied upon structural homology calculations using threading methods based on alignment of the sequence against a library of solved three-dimensional protein structures, for prediction of the LCAT fold. We propose that LCAT, like lipases, belongs to the alpha/beta hydrolase fold family, and that the central domain of LCAT consists of seven conserved parallel beta-strands connected by four alpha-helices and separated by loops. We used the conserved features of this protein fold for the prediction of functional domains in LCAT, and carried out site-directed mutagenesis for the localization of the active site residues. The wild-type enzyme and mutants were expressed in Cos-1 cells. LCAT mass was measured by ELISA, and enzymatic activity was measured on recombinant HDL, on LDL and on a monomeric substrate. We identified D345 and H377 as the catalytic residues of LCAT, together with F103 and L182 as the oxyanion hole residues. In analogy with lipases, we further propose that a potential "lid" domain at residues 50-74 of LCAT might be involved in the enzyme-substrate interaction. Molecular modeling of human LCAT was carried out using human pancreatic and Candida antarctica lipases as templates. The three-dimensional model proposed here is compatible with the position of natural mutants for either LCAT deficiency or Fish-eye disease. It enables moreover prediction of the LCAT domains involved in the interaction with the phospholipid and cholesterol substrates.

L29 ANSWER 10 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 4

ACCESSION NUMBER: 1998:811251 CAPLUS Full-text
DOCUMENT NUMBER: 130:164804
TITLE: Fluorescence study of fungal lipase from Humicola lanuginosa
AUTHOR(S): Stobiecka, A.; Wysocki, S.; Brzozowski, A. M.

CORPORATE SOURCE: Inst. General Food Chem., Tech. Univ., Lodz, 90-924, Pol.
SOURCE: Journal of Photochemistry and Photobiology, B: Biology (1998), 45(2-3), 95-102
CODEN: JPPBEG; ISSN: 1011-1344
PUBLISHER: Elsevier Science S.A.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Time-resolved and steady-state fluorescence quenching measurements have been performed to study two different conformations of the fungal lipase from Humicola lanuginosa. The intrinsic fluorescence of tryptophan Trp89 residue, located in the 'lid' region, has been used as a probe for the dynamics of protein. The native ('closed-lid') form of the enzyme has been found to decay as a triple exponential with time consts. and relative contributions of 5.4 ns (74.3%), 2.2 ns (20.4%) and 0.4 ns (5.3%). A comparison of recovered decay parameters obtained for native and mutated H. lanuginosa lipase shows that Trp89 contributes about 61% to the class of emitting species with the lifetime of 5.4 ns. The fluorescence quenching data show that three out of four tryptophans (i.e., 117, 221 and 260 residues) within H. lanuginosa lipase are totally quenchable by acrylamide while completely inaccessible to iodide. On the contrary, the Trp89 residue is available for both quenchers. Using steady-state iodide fluorescence quenching data and the fluorescence-quenching-resolved-spectra (FQRS) method, the total emission spectrum of the native lipase has been decomposed into two spectral components. One of them, unquenchable by iodide, has a maximum of fluorescence emission at 330 nm and the second one, exposed to the solvent, emits at 338 nm. The resolved spectrum of the redder component corresponds to the Trp89 residue, which participates in about 65% of the total H. lanuginosa emission. The dynamic Stern-Volmer quenching consts. calculated for both native ('closed-lid') and inhibited ('open-lid') lipase are 2.71 and 4.49 M-1, resp. The values obtained indicate that Trp89 is not deeply buried in the protein matrix. Our results suggest that distinct configurations of fungal lipase can be monitored using the fluorescence of the Trp89 residue located in the 'lid'-helix which participates in an interfacial activation of the enzyme.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 11 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:587613 SCISEARCH Full-text
THE GENUINE ARTICLE: 106NT
TITLE: Anatomy of lipase binding sites: the scissile fatty acid binding site
AUTHOR: Pleiss J; Fischer M; Schmid R D (Reprint)
CORPORATE SOURCE: Univ Stuttgart, Inst Tech Biochem, Allmandring 31, D-70569 Stuttgart, Germany (Reprint); Univ Stuttgart, Inst Tech Biochem, D-70569 Stuttgart, Germany
COUNTRY OF AUTHOR: Germany
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No. 1-2, pp. 67-80.
ISSN: 0009-3084.
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 41
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Shape and physico-chemical properties of the scissile fatty acid binding sites of six lipases and two serine esterases were analyzed and compared in order to understand the molecular basis of substrate specificity. All eight serine esterases and lipases have similar architecture and catalytic mechanism of ester hydrolysis, but different substrate specificities for the acyl moiety. Lipases and esterases differ in the geometry of their binding sites, lipases have a large, hydrophobic scissile fatty acid binding site, esterases like acetylcholinesterase and bromoperoxidase have a small acyl binding pocket, which fits exactly to their favorite substrates. The lipases were subdivided into three sub-groups: (1) lipases with a hydrophobic, crevice-like binding site located near the protein surface (lipases from Rhizomucor and Rhizopus); (2) lipases with a funnel-like binding site (lipases from Candida antarctica, Pseudomonas and mammalian pancreas and cutinase); and (3) lipases with a tunnel-like binding site (lipase from Candida rugosa). The length of the scissile fatty acid binding site varies considerably among the lipases between 7.8 Angstrom in cutinase and 22 Angstrom in Candida rugosa and Rhizomucor miehei lipase. Location and properties of the scissile fatty acid binding sites of all lipases of known structure were characterized. Our model also identifies the residues which mediate chain length specificity and thus may guide protein engineering of lipases for changed chain length specificity. The model was supported by published experimental data on the chain length specificity profile of various lipases and on mutants of fungal lipases with changed fatty acid chain length specificity.

L29 ANSWER 12 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:587612 SCISEARCH Full-text
THE GENUINE ARTICLE: 106NT
TITLE: Insights into the molecular basis for fatty acyl specificities of lipases from Geotrichum candidum and Candida rugosa
AUTHOR: Holmquist M (Reprint)
CORPORATE SOURCE: Royal Inst Technol, Dept Biochem & Biotechnol, SE-10044 Stockholm, Sweden (Reprint)
COUNTRY OF AUTHOR: Sweden
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No. 1-2, pp. 57-66.
ISSN: 0009-3084.
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 49
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Despite immense progress in our comprehension of lipase structure and function during the past decade, the basis for lipase acyl specificities has remained poorly understood. This review summarizes some recent advances in the understanding at the molecular-level of substrate acyl recognition by two members in a group of large (M-w similar to 60 kDa) microbial lipases. Two aspects of acyl specificity will be focused upon. (1) The unique preference of a fungal Geotrichum candidum lipase for long-chain cis (Delta-9) unsaturated fatty acid moieties in the substrate. Mutational analysis of this lipase identified residues essential for its anomalous acyl preference. This information highlighted for the first time parts in the lipase molecule involved in substrate acyl differentiation. These results are discussed in the context of the 3D-structure of a G. candidum lipase isoenzyme and structures of the related Candida rugosa lipase in complex with inhibitors. (ii) The mechanism by which the yeast C. rugosa lipase discriminates between enantiomers of a substrate with a chiral acyl moiety. Molecular modeling in combination with substrate engineering and kinetic analyses, identified two alternative substrate binding modes. This allowed for the proposal of a molecular mechanism explaining how long-chain alcohols can act as enantioselective inhibitors of this enzyme. A picture is thus beginning to emerge of the interplay between lipase structure and fatty acyl specificity. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.

L29 ANSWER 13 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 5

ACCESSION NUMBER: 1998:455633 CAPLUS Full-text
DOCUMENT NUMBER: 129:213493
TITLE: The decay of the fluorescence anisotropy of tryptophan residues in fungal lipase from Humicola lanuginosa
AUTHOR(S): Stobiecka, A.; Wysocki, S.
CORPORATE SOURCE: Institute General Food Chemistry, Technical University Lodz, Lodz, 90924, Pol.
SOURCE: Journal of Radioanalytical and Nuclear Chemistry (1998), 232(1-2), 43-48
CODEN: JRNCDM; ISSN: 0236-5731
PUBLISHER: Elsevier Science S.A.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Pulse nanosecond fluorescence anisotropy decay was used to study the mobility of Trp residues within fungal lipase from Humicola lanuginosa. The decay of emission anisotropy of protein in native, inhibited, and mutated form was investigated in buffered water and 50% volume/volume glycerol solns. The rotational motions of the lipase were analyzed in terms of 2 different kinetic models. The fluorescence emission anisotropy decay can best be described with 2 rotational correlation times : 0.63 and 5.45 ns in water and 0.98 and 10.70 ns and in 50% volume/volume glycerol solution The decay of inhibited and mutated H. lanuginosa lipase showed a similar biexponential character. These results are interpreted in terms of local or segmental motion arising from a mass of about 1083 Da which corresponds to the 'lid'-helix fragment of the enzyme.

L29 ANSWER 14 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:587609 SCISEARCH Full-text
THE GENUINE ARTICLE: 106NT

TITLE: Biochemical properties of staphylococcal (phospho) lipases
AUTHOR: Simons J W F A; Gotz F; Egmond M R; Verheij H M (Reprint)
CORPORATE SOURCE: Univ Utrecht, Inst Biomembranes, Ctr biomembranes & Lipid Enzymol, Dept Enzymol & Prot Engn, POB 80054, Padualaan 8, NL-3508 TB Utrecht, Netherlands (Reprint); Univ Utrecht, Inst Biomembranes, Ctr biomembranes & Lipid Enzymol, Dept Enzymol & Prot Engn, NL-3508 TB Utrecht, Netherlands; Univ Tubingen, Tubingen, Germany
COUNTRY OF AUTHOR: Netherlands; Germany
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No. 1-2, pp. 27-37.
ISSN: 0009-3084.
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 37
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Various staphylococci secrete lipases which require calcium ions for activity, and have profound preferences for substrates with different chain lengths. The lipase from Staphylococcus hyicus is exceptional since it has higher phospholipase than lipase activity. This paper gives an overview of the biochemical properties: of these enzymes. It appears that chain length selectivity of these enzymes resides in the acylation step. Interfaces mainly influence the acylation step. Calcium ions do not influence the rate of acylation or deacylation although stabilise the enzyme against denaturation. Molecular modelling based on the X-ray structure of Pseudomonas glumae lipase was used to construct a model of the staphylococcal lipases. With this model the position of serveral residues involved in stubstrate selectivity was predicted. Moreover, a sequence element could be assigned that may function as the so-called lid domain in staphylococcal lipases. Sequence alignment of four staphylococcal lipases, and lipases from P. glumae and Bacillus thermocatenulatus identified several potential calcium ligands, one of which was verified by site directed mutagensesis. It is concluded that stabilisation of lipases by calcium ions might be a more general phenomenon than recognized so far. (C) 1998 Elsevier Science Ireland Ltd.

L29 ANSWER 15 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:757085 CAPLUS Full-text
DOCUMENT NUMBER: 128:24317
TITLE: Detergent compositions containing a specific lipolytic enzyme and alkyl polyglycoside surfactant
INVENTOR(S): Baeck, Andre Cesar; Kasturi, Chandrika
PATENT ASSIGNEE(S): Procter and Gamble Company, USA; Baeck, Andre Cesar; Kasturi, Chandrika
SOURCE: PCT Int. Appl., 74 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9743377	A1	19971120	WO 1996-US7088	19960515
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9658618	A	19971205	AU 1996-58618	19960515
EP 912683	A1	19990506	EP 1996-920251	19960515
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
BR 9612608	A	19990720	BR 1996-12608	19960515
PRIORITY APPLN. INFO.:			WO 1996-US7088 W	19960515

AB The present invention relates to detergent compns. containing a variants of the native lipolytic enzyme producible by Humicola lanuginosa and Thermomyces lanuginosus or by cloning and expressing the gene responsible for producing the variants into a host organism and an ROGx surfactant (R = C10-16 alkyl, G = reducing saccharide containing 5-6 C atoms, x = 1-3). Such compns. provide

improved overall detergency performance: reduced redeposition of greasy/oily substances on fabrics, dishware and hard surfaces thereby improving whiteness maintenance, cleaning, spotting, filming and stain removal performances.

L29 ANSWER 16 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:757082 CAPLUS Full-text
DOCUMENT NUMBER: 128:36417
TITLE: Detergent compositions comprising specific lipolytic enzyme and a soil release polymer
INVENTOR(S): Baeck, Andre Cesar; Kasturi, Chandrika
PATENT ASSIGNEE(S): Procter and Gamble Company, USA; Baeck, Andre Cesar; Kasturi, Chandrika
SOURCE: PCT Int. Appl., 77 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9743374	A1	19971120	WO 1996-US7085	19960515
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2254919	A1	19971120	CA 1996-2254919	19960515
AU 9657957	A	19971205	AU 1996-57957	19960515
BR 9612614	A	19990720	BR 1996-12614	19960515
EP 931134	A1	19990728	EP 1996-914666	19960515
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				

PRIORITY APPLN. INFO.: WO 1996-US7085 A 19960515

AB Lipolytic enzymes are variants of the native lipolytic enzymes producible by Humicola lanuginosa and Thermomyces lanuginosus. A soil release polymer is selected from sulfonated poly(ethoxy)/(propoxy)-end capped ester oligomers and/or short block polymers manufactured from di-Me terephthalate (I), 1,2-propylene glycol (II), Me-capped polyethylene glycol and/or short block polymers manufactured from I, II, and sulfoethoxylates. Thus, a granular detergent contained a Na linear C12-alkyl benzenesulfonate 22, a phosphate 23, a carbonate 23, a silicate 14, Zeolite A 8.2, Dequest 2060 0.4, Na sulfate 5.5, amylase 0.005, protease 0.01, pectinase 0.02, Lipolase Ultra 0.005, a soil release polymer 0.2, cellulase 0.001, and water and minors to 100%.

L29 ANSWER 17 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:220628 CAPLUS Full-text
DOCUMENT NUMBER: 126:208956
TITLE: Recombinant lipases with C- and/or N-terminal extensions and their use in detergents
INVENTOR(S): Fuglsang, Claus Crone; Okkels, Jens Sigurd; Pertersen, Dorte Aaby; Patkar, Shamkant Anant; Thellersen, Marianne; Vind, Jesper; Halkier, Torben; Joergensen, Steen Troels; et al.
PATENT ASSIGNEE(S): Novo Nordisk A/s, Den.; Fuglsang, Claus Crone; Okkels, Jens Sigurd; Pertersen, Dorte Aaby; Patkar, Shamkant Anant; Thellersen, Marianne; Vind, Jesper; Halkier, Torben
SOURCE: PCT Int. Appl., 197 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9704078	A1	19970206	WO 1996-DK321	19960712
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				

RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA

AU 9664140	A	19970218	AU 1996-64140		19960712
CN 1193346	A	19980916	CN 1996-196371		19960712
PRIORITY APPLN. INFO.:			DK 1995-832	A	19950714
			DK 1995-1013	A	19950913
			DK 1995-1096	A	19950929
			DK 1995-1306	A	19951121
			US 1996-11634P	P	19960214
			DK 1996-372	A	19960401
			US 1996-20461P	P	19960507
			WO 1996-DK321	W	19960712

AB The invention relates to a modified enzyme with lipolytic activity recovered from a filamentous fungi or a bacteria having one or more peptide addns. at the N-terminal and/or the C-terminal ends in comparison to the parent enzyme. The peptide addns. significantly improve the washing performance of the lipase. Further, the invention relates to a DNA sequence encoding said modified enzyme, a vector comprising said DNA sequence, a host cell harboring said DNA sequence or said vector, and a process for producing said modified enzyme with lipolytic activity. The lipase variants are useful in detergent compns. Numerous lipase variants containing substitution mutations and C- and/or N-terminal addns. were prepared with recombinant Aspergillus oryzae or with Escherichia coli. The addition of SPIRR to the N-terminus of Humicola lanuginosa lipase increased the wash performance relative to the parent enzyme twofold.

L29 ANSWER 18 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:897558 SCISEARCH Full-text
THE GENUINE ARTICLE: YJ709
TITLE: Identification of residues essential for differential fatty acyl specificity of Geotrichum candidum lipases I and II
AUTHOR: Holmquist M (Reprint); Tessier D C; Cygler M
CORPORATE SOURCE: NATL RES COUNCIL CANADA, BIOTECHNOL RES INST, MONTREAL, PQ H4P 2R2, CANADA; ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, S-10044 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: CANADA; SWEDEN
SOURCE: BIOCHEMISTRY, (2 DEC 1997) Vol. 36, No. 48, pp. 15019-15025.
ISSN: 0006-2960.
PUBLISHER: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 44
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The fungus Geotrichum candidum produces two lipase isoenzymes, GCL I and GCL II, with distinct differences in substrate specificity despite their 86% identical primary structure, GCL I prefers ester substrates with long-chain cis (Delta-9) unsaturated fatty acid moieties, whereas GCL II also accepts medium-length (C8-C14) acyl moieties in the substrate. To reveal structural elements responsible for differences in substrate differentiating ability of these isoenzymes, we designed, expressed, and characterized 12 recombinant lipase variants. Three chimeric lipases containing unique portions of the N-terminal and the C-terminal part of GCL I and GCL II, respectively, were constructed and enzymatically characterized. Activities were measured against mixed triglyceride-poly(dimethyl siloxane) particles. Our results indicate that residues within sequence positions 349-406 are essential for GCL I's high triolein/trioctanoin activity ratio of 20. The substitution of that segment in the specific GCL I to the corresponding residues in the nonspecific GCL II, resulted in an enzyme with a triolein/trioctanoin activity ratio of 1.4, identical to that of GCL IL. The reverse mutation in GCL II increased its specificity for triolein by a factor of 2, thus only in part restoring the high specificity seen with GCL I. In further experiments, the point mutations at the active site entrance of the GCL I, Leu358Phe and Ile357Ala/Leu358Phe, lowered the triolein/trioctanoin activity ratio from 20 to 4 and 2.5, respectively. The substitutions Cys379Phe/Ser380Tyr at the bottom of the active site cavity of GCL I decreased its specificity to a value of 3.6, Measurements of lipase activity with substrate particles composed of pure triglycerides or ethyl esters of oleic and octanoic acids resulted in qualitatively similar results as reported above. Our data reveal for the first time the identity of residues essential for the unusual substrate preference of GCL I and show that the anatomy, both at the entrance and the bottom of the active site cavity, plays a key role in substrate discrimination.

L29 ANSWER 19 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 6

ACCESSION NUMBER: 1997:372120 CAPLUS Full-text

DOCUMENT NUMBER: 126:327220
TITLE: Comparison of the adsorption and activity of lipases from Humicola lanuginosa and Candida antarctica on solid surfaces
AUTHOR(S): Wannerberger, Kristin; Arnebrant, Thomas
CORPORATE SOURCE: Department of Food Technology, University of Lund, Lund, S-221 00, Swed.
SOURCE: Langmuir (1997), 13(13), 3488-3493
CODEN: LANGD5; ISSN: 0743-7463
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The adsorption of lipases from H. lanuginosa, the wild type (WT) and a mutant with increased hydrophobicity in the active site region, and lipase B from C. antarctica to solid surfaces was studied by in situ ellipsometry. In addition, the activity of the adsorbed lipase was measured in situ and from the different surface concns., the specific activity was calculated Concentration- and temperature-dependence as well as the influence of surface wettability was studied using silica surfaces with varying degrees of methylation. The higher hydrophobicity of the mutant compared to the WT resulted in increased amts. adsorbed, no desorbable fraction during rinsing, and absence of an initial maximum in adsorbed amount (as seen for the WT) at higher concns. No temperature dependence for the mutant could be observed This was in contrast to the WT where both the plateau value of the adsorbed amount and the activity decreased with increasing temperature The influence of surface wettability was similar for both featuring a decreased adsorbed amount and increased specific activity with increasing wettability. The amount adsorbed of the Candida lipase was significantly higher at all concns., compared to the other lipases, and the activity was very low, indicating adsorption with the active site region directed toward the surface. The surface wettability did not affect the activity of Candida lipase.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L29 ANSWER 20 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:548646 CAPLUS Full-text
DOCUMENT NUMBER: 127:189671
TITLE: Biotechnology in detergents and cosmetics
AUTHOR(S): Ito, Susumu
CORPORATE SOURCE: Tochigi Res. Lab., Kao Corp., Tochigi, 321-34, Japan
SOURCE: Baiosaiensu to Indasutori (1997), 55(8), 541-545
CODEN: BIDSE6; ISSN: 0914-8981
PUBLISHER: Baioindasutori Kyokai
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese

AB A review with 18 refs. on site-directed mutation of subtilisin suitable for detergents, heat-stable mutation of Bacillus licheniformis amylase, amylopullulanase, site-directed mutation of α-amylase to improve durability against chelating agents and heat, alkaline cellulase of alkalophilic Bacillus, cellulase of Aspergillus oryzae, production of lipase of Humicola lanuginosa by A. oryzae as host organism, catalytic site of Mocor miehei enzyme, raw materials for cosmetics, sophorolipid, kojic acid, hyaluronate, γ-linolenate, shikonin, and tuberose polysaccharide.

L29 ANSWER 21 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1997:250761 SCISEARCH Full-text
THE GENUINE ARTICLE: WP714
TITLE: Computational studies of the activation of lipases and the effect of a hydrophobic environment
AUTHOR: Peters G H (Reprint); Toxvaerd S; Olsen O H; Svendsen A
CORPORATE SOURCE: UNIV COPENHAGEN, HC ORSTED INST, DEPT CHEM 3, UNIV PK 5, DK-2100 COPENHAGEN O, DENMARK (Reprint); NOVO NORDISK AS, DK-2880 BAGSVAERD, DENMARK
COUNTRY OF AUTHOR: DENMARK
SOURCE: PROTEIN ENGINEERING, (FEB 1997) Vol. 10, No. 2, pp. 137-147.
ISSN: 0269-2139.
PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD, ENGLAND OX2 6DP.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 69
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have investigated the activation pathway of three wild type lipases and three mutants using molecular dynamics techniques combined with a constrained mechanical protocol. The

activation of these lipases involves a rigid body hinge-type motion of a single helix, which is displaced during activation to expose the active site and give access to the substrate. Our results suggest that the activation of lipases is enhanced in a hydrophobic environment as is generally observed in experiments. The energy gain upon activation varies between the different lipases and depends strongly on the distribution of the charged residues in the activating loop region. In a low dielectric constant medium (such as a lipid environment), the electrostatic interactions between the residues located in the vicinity of the activating loop (lipid contact zone) are dominant and determine the activation of the lipases. Calculations of the pK(a)s qualitatively indicate that some titratable residues experience significant pK shifts upon activation. These calculations may provide sufficient details for an understanding of the origin and magnitude of a given electrostatic effect and may provide an avenue for exploring the activation pathway of lipases.

L29 ANSWER 22 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:170960 SCISEARCH Full-text
THE GENUINE ARTICLE: WJ876
TITLE: Altered acyl chain length specificity of Rhizopus delemar lipase through mutagenesis and molecular modeling
AUTHOR: Klein R R (Reprint); King G; Moreau R A; Haas M J
CORPORATE SOURCE: USDA ARS, ERRC, 600 E MERMAID LANE, WYNDMOOR, PA 19038 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: LIPIDS, (FEB 1997) Vol. 32, No. 2, pp. 123-130.
ISSN: 0024-4201.
PUBLISHER: AMER OIL CHEMISTS SOC, 1608 BROADMOOR DRIVE, CHAMPAIGN, IL 61821-0489.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 27
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The acyl binding site of Rhizopus delemar prolipase and mature lipase was altered through site-directed mutagenesis to improve lipase specificity for short- or medium-chain length fatty acids. Computer-generated structural models of R. delemar lipase were used in mutant protein design and in the interpretation of the catalytic properties of the resulting recombinant enzymes. Molecular dynamics simulations of the double mutant, val209trp + phe112trp, predicted that the introduction of trp112 and trp209 in the acyl binding groove would sterically hinder the docking of fatty acids longer than butyric acid. Assayed against a mixture of triacylglycerol substrates, the val209trp + phe112trp mature lipase mutant showed an 80-fold increase in the hydrolysis of tributyrin relative to the hydrolysis of tricaprylin while no triolein hydrolysis was detected. By comparison, the val94Trp mutant, predicted to pose steric or geometric constraints for docking fatty acids longer than caprylic acid in the acyl binding groove, resulted in a modest 1.4-fold increase in tricaprylin hydrolysis relative to the hydrolysis of tributyrin. Molecular models of the double mutant phe95asp + phe214arg indicated the creation of a salt bridge between asp95 and arg214 across the distal end of the acyl binding groove. When challenged with a mixture of triacylglycerols, the phe95asp + phe214arg substitutions resulted in an enzyme with 3-fold enhanced relative activity for tricaprylin compared to triolein, suggesting that structural determinants for medium-chain length specificity may reside in the distal end of the acyl binding groove. Attempts to introduce a salt bridge within 8 Angstrom of the active site by the double mutation leu146lys + ser1 15asp destroyed catalytic activity entirely. Similarly, the substitution of polar Gln at the rim of the acyl binding groove for phe112 largely eliminated catalytic activity of the lipase.

L29 ANSWER 23 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:470292 SCISEARCH Full-text
THE GENUINE ARTICLE: XF115
TITLE: Computer-aided modelling of stereoselective triglyceride hydrolysis catalyzed by Rhizopus oryzae lipase
AUTHOR: Holzwarth H C (Reprint); Pleiss J; Schmid R D
CORPORATE SOURCE: UNIV STUTTGART, INST TECH BIOCHEM, D-70569 STUTTGART, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: JOURNAL OF MOLECULAR CATALYSIS B-ENZYMATIC, (10 JUN 1997) Vol. 3, No. 1-4, pp. 73-82.
ISSN: 1381-1177.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM,

NETHERLANDS.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: English
REFERENCE COUNT: 28
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Lipase from Rhizopus oryzae catalyzes the stereoselective hydrolysis of triglycerides and analogues. Stereopreference and degree of enantiomeric excess of the product varies with the structure of the substrate: trioctanoylglycerol ('ester') and the sn-2 analogues 2-X-1,3-dioctanoylpropandiol, where 2-X = 2-O-octyl ('ether') and 2-hexyl('alkane'), are preferentially hydrolyzed at sn-1, substitution by 2-phenyl ('phenyl') reverses the stereopreference to sn-3. We have modelled the stereoselectivity of Rhizopus oryzae lipase by docking the tetrahedral intermediates of these substrates in two orientations, appropriate to hydrolysis at sn-1 or sn-3, respectively. The initial complexes were further relaxed by molecular dynamics simulations. The favoured orientation of a substrate is characterized by three factors: (1) The substrate fits well into the binding site. The glycerol backbone is relaxed and the sn-2 chain points to a well defined hydrophobic binding site. (2) The oxyanion is stabilized by an extra hydrogen bond from the side chain of Thr 83. (3) The substrate lacks repulsive interactions with protein side chains, especially of Leu 258.
Our model is consistent with experimental data and explains qualitatively the ranking of four different substrates with regard to stereoselectivity. It can be used to design lipase mutants with modified stereoselectivity.

L29 ANSWER 24 OF 37 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 97042947 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 8888145
TITLE: The consequences of engineering an extra disulfide bond in the Penicillium camembertii mono- and diglyceride specific lipase.
AUTHOR: Yamaguchi S; Takeuchi K; Mase T; Oikawa K; McMullen T; Derewenda U; McElhaney R N; Kay C M; Derewenda Z S
CORPORATE SOURCE: Tsukuba Research Laboratories, Amano Pharmaceutical Co. Ltd., Japan.
SOURCE: Protein engineering, (1996 Sep) Vol. 9, No. 9, pp. 789-95.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 14 May 1997
Last Updated on STN: 14 May 1997
Entered Medline: 6 May 1997

AB The extracellular lipase from Penicillium camembertii has unique substrate specificity restricted to mono- and diglycerides. The enzyme is a member of a homologous family of lipases from filamentous fungi. Four of these proteins, from the fungi Rhizomucor miehei, Humicola lanuginosa, Rhizopus delemar and P. camembertii, have had their structures elucidated by X-ray crystallography. In spite of pronounced sequence similarities the enzymes exhibit significant differences. For example, the thermostability of the P. camembertii lipase is considerably lower than that of the H. lanuginosa enzyme. Since only the P. camembertii enzyme lacks the characteristic long disulfide bridge, corresponding to Cys22-Cys268 in the H. lanuginosa lipase, we have engineered this disulfide into the former enzyme in the hope of obtaining a significantly more stable fold. The properties of the double mutant (Y22C and G269C) were assessed by a variety of biophysical techniques. The extra disulfide link was found to increase the melting temperature of the protein from 51 to 63 degrees C. However, no difference is observed under reducing conditions, indicating an intrinsic instability of the new disulfide. The optimal temperature for catalytic activity decreased by 10 degrees C and the optimum pH was shifted by 0.7 units to more acidic.

L29 ANSWER 25 OF 37 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 97015915 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 8862552
TITLE: The role of Glu87 and Trp89 in the lid of Humicola lanuginosa lipase.
AUTHOR: Martinelle M; Holmquist M; Clausen I G; Patkar S; Svendsen A; Hult K
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
SOURCE: Protein engineering, (1996 Jun) Vol. 9, No. 6, pp. 519-24.

Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 28 Jan 1997
Last Updated on STN: 29 Jan 1999
Entered Medline: 9 Dec 1996

AB The importance of Glu87 and Trp89 in the lid of Humicola lanuginosa lipase for the hydrolytic activity at the water/lipid interface was investigated by site-directed mutagenesis. It was found that the effect on the hydrolytic activity upon the replacement of Trp89 with Phe, Leu, Gly or Glu was substrate dependent. The Trp89 mutants displayed an altered chain length specificity towards triglycerides, with a higher relative activity towards triacetin and trioctanoin compared with tributyrin. Trp89 was shown to be less important in the hydrolysis of vinyl esters compared with ethyl esters and triglycerides. An exclusive effect on the acylation reaction rate by the mutation of Trp89 was consistent with the data. It is suggested that Trp89 is important in the process of binding the acyl chain of the substrate into the active site for optimal acylation reaction rate. The Trp89Phe mutation resulted in an increased hydrolytic activity towards 2-alkylalkanoic acid esters. This is suggested to be due to reduction of unfavourable van der Waals contacts between Trp89 and the 2-substituent of the substrate. Thus, in contrast to natural substrates, Trp89 has a negative impact on the catalytic efficiency when substrates with bulky acyl chains are used. In contrast to the Trp89 mutations, the effect on the hydrolytic activity of the Glu87Ala mutation was almost substrate independent, 35-70% activity of wild-type lipase. A reduction of both the acylation and deacylation reaction was consistent with the data.

L29 ANSWER 26 OF 37 LIFESCI COPYRIGHT 2007 CSA on STN
ACCESSION NUMBER: 1998:29753 LIFESCI Full-text
TITLE: Acyl glycerol hydrolases: Inhibitors, interface and catalysis
AUTHOR: Cambillau, C.; Longhi, S.; Nicolas, A.; Martinez, C.
CORPORATE SOURCE: Architecture et Fonction des Macromolecules Biologiques, UPR 9039, CNRS, IFRI, 31 Chemin Joseph Aiguier, 13402 Marseille, Cedex 20, France
SOURCE: CURR. OPIN. STRUCT. BIOL., (19960800) vol. 6, no. 4, pp. 449-455.
ISSN: 0959-440X.
DOCUMENT TYPE: Journal
TREATMENT CODE: General Review
FILE SEGMENT: K
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The last five years have witnessed the solution of a large number of lipase structures, which has led, among other insights, to the structural interpretation of the interfacial activation phenomenon in terms of 'lid' opening. This interpretation has been extended this year to include phospholipase A2. Recent structural studies on lipases have provided data on the detailed mechanisms underlying the behaviour of lipases: how they bind to inhibitors or substrates, and what interactions occur between their hydrophobic face and hydrophobic molecules, for example. In addition, studies on cutinase point mutants have shed some light on the role of the oxyanion hole in lipolytic catalysis.

L29 ANSWER 27 OF 37 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1996:113975 BIOSIS Full-text
DOCUMENT NUMBER: PREV199698686110
TITLE: Contribution of cutinase serine 42 side chain to the stabilization of the oxyanion transition state.
AUTHOR(S): Nicolas, Anne; Egmond, Maarten; Verrips, C. Theo; De Vlieg, Jakob; Longhi, Sonia; Cambillau, Christian [Reprint author]; Martinez, Chrislaine
CORPORATE SOURCE: Lab. Cristallographic Cristallisation des Macromolecules Biol., URA1296-CNRS, IFR1, 31 Chemin J. Aiguier, 13402 Marseille cedex 09, France
SOURCE: Biochemistry, (1996) Vol. 35, No. 2, pp. 398-410.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Mar 1996
Last Updated on STN: 10 Jun 1997

AB Cutinase from the fungus Fusarium solani pisi is a lipolytic enzyme able to hydrolyze both aggregated and soluble substrates. It therefore provides a powerful toot for probing the mechanisms underlying lipid hydrolysis. Lipolytic enzymes have a catalytic machinery similar to

those present in serine proteinases. It is characterized by the triad Ser, His, and Asp (Glu) residues, by an oxyanion binding site that stabilizes the transition state via hydrogen bonds with two main chain amide groups, and possibly by other determinants. It has been suggested on the basis of a covalently bond inhibitor that the cutinase oxyanion hole may consist not only of two main chain amide groups but also of the Ser42 O-gamma side chain. Among the esterases and the serine and the cysteine proteases, only Streptomyces scabies esterase, subtilisin, and papain, respectively, have a side chain residue which is involved in the oxyanion hole formation. The position of the cutinase Ser42 side chain is structurally conserved in Rhizomucor miehei lipase with Ser82 O-gamma, in Rhizopus delemar lipase with Thr83 O-gamma-1, and in Candida antartica B lipase with Thr40 O-gamma-1. To evaluate the increase in the tetrahedral intermediate stability provided by Ser42 O-gamma, we mutated Ser42 into Ala. Furthermore, since the proper orientation of Ser42 O-gamma is directed by Asn84, we mutated Asn84 into Ala, Leu, Asp, and Trp, respectively, to investigate the contribution of this indirect interaction to the stabilization of the oxyanion hole. The S42A mutation resulted in a drastic decrease in the activity (450-fold) without significantly perturbing the three-dimensional structure. The N84A and N84L mutations had milder kinetic effects and did not disrupt the structure of the active site, whereas the N84W and N84D mutations abolished the enzymatic activity due to drastic steric and electrostatic effects, respectively.

L29 ANSWER 28 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1996:404098 CAPLUS Full-text
DOCUMENT NUMBER: 125:133994
TITLE: A URA3-promoter deletion in a pYES vector increases the expression level of a fungal lipase in Saccharomyces cerevisiae
AUTHOR(S): Okkels, Jens Sigurd
CORPORATE SOURCE: Novo Nordisk, Copenhagen, DK-2100, Den.
SOURCE: Annals of the New York Academy of Sciences (1996), 782(Recombinant DNA Biotechnology III), 202-207
CODEN: ANYAA9; ISSN: 0077-8923
PUBLISHER: New York Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Expression of a lipase gene from Humicola lanuginosa in Saccharomyces cerevisiae is increased, allowing large-scale purification A simple deletion of the URA3 promoter from a S. cerevisiae expression plasmid was performed. The promoter-deleted plasmid has an increased expression level of fungal lipase gene. The deletion probably causes a poor expression of the URA3 selection marker, probably resulting in a higher copy number per cell of the plasmid. This higher copy number can increase the transcript level per cell and there by the expression level. In the case of the fungal lipase gene, the expression level with defined inoculum is increased at least three-fold. A part of the 2-μm origin of the pYES type plasmid was also deleted by the URA3 promoter deletion without affecting transformation frequency. The URA3 promoter can easily be deleted from most pYES type plasmids, since most of these plasmids only contain the two PstI sites that remove the whole URA3 promoter.

L29 ANSWER 29 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 9
ACCESSION NUMBER: 1996:490978 CAPLUS Full-text
DOCUMENT NUMBER: 125:215670
TITLE: Lipases from Humicola lanuginosa adsorbed to hydrophobic surfaces - desorption and activity after addition of surfactants
AUTHOR(S): Wannerberger, Kristin; Arnebrant, Thomas
CORPORATE SOURCE: Department of Food Technology, University of Lund, Box 124, Lund, S-22100, Swed.
SOURCE: Colloids and Surfaces, B: Biointerfaces (1996), 7(3/4), 153-164
CODEN: CSBBEQ; ISSN: 0927-7765
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The adsorption of lipase to methylated silica and its subsequent removal upon sequential addition of surfactants were measured in situ by ellipsometry. In addition, the specific activity of adsorbed lipase without, and after, exposure to surfactants was determined Two lipases from Humicola lanuginosa were studied, the wild-type (WT) and a mutant which was modified to increase the hydrophobicity of the active-site region. The surfactants used were SDS, C12E5 and sodium dodecanoate and they were all studied in the concentration range ≤ their critical micelle concentration (cmc). In all expts. a non-desorbable fraction remained at the surface. This fraction was larger, however, for the WT compared to the mutant. The concentration, expressed as a fraction of the cmc, of surfactant inducing the most pronounced desorption was found to vary among the surfactants. Desorption occurred at increasing fractions of the cmc in the order C12E5, sodium dodecanoate and SDS. This was found for both the WT and the mutant. No significant effect of C12E5 or SDS on the specific activities could be observed while the exposure to sodium dodecanoate markedly increased the specific activity of both lipases.

L29 ANSWER 30 OF 37 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 95242058 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 7536956
TITLE: The importance of non-charged amino acids in antibody binding to Humicola lanuginosa lipase.
AUTHOR: Naver H; Lovborg U
CORPORATE SOURCE: Novo Nordisk Industrial Biotechnology, Copenhagen, Denmark.
SOURCE: Scandinavian journal of immunology, (1995 May) Vol. 41, No. 5, pp. 443-8.
Journal code: 0323767. ISSN: 0300-9475.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 5 Jun 1995
Last Updated on STN: 29 Jan 1999
Entered Medline: 25 May 1995

AB The antigenicity of 36 Humicola lanuginosa lipase (HL) variants, generated by site directed mutagenesis, was compared with that of the unchanged enzyme. Polyclonal antibodies raised against variant lipases were investigated and compared with the antibodies raised against the wild type lipase in an ELISA competition assay. The results showed that exchange of charged amino acids with polar residues in surface epitopes of HL, results in a tighter binding of the antibody to the epitope. Four amino acids (Trp at position 89, Asp at positions 96 and 254 and Phe at position 211) were found to be essential for antibody binding in each their epitope of the wild type enzyme.

L29 ANSWER 31 OF 37 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 95391076 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 7662109
TITLE: Probing a functional role of Glu87 and Trp89 in the lid of Humicola lanuginosa lipase through transesterification reactions in organic solvent.
AUTHOR: Holmquist M; Clausen I G; Patkar S; Svendsen A; Hult K
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
SOURCE: Journal of protein chemistry, (1995 May) Vol. 14, No. 4, pp. 217-24.
Journal code: 8217321. ISSN: 0277-8033.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 20 Oct 1995
Last Updated on STN: 29 Jan 1999
Entered Medline: 10 Oct 1995

AB To reveal the functional role of Glu87 and Trp89 in the lid of Humicola lanuginosa lipase, site-directed mutagenesis at Glu87 and Trp89 was carried out. The catalytic performance of wild-type and mutated lipases was studied in transesterification reactions in cyclohexane at a controlled water activity. Two different acyl donors were used in the investigation: tributyrin, a natural substrate for a lipase, and vinyl butyrate, an activated ester suitable for fast and efficient lipase-catalyzed transformations in preparative organic synthesis. As acyl acceptor 1-heptanol was used. The Glu87Ala mutation decreased the Vmax,app value with tributyrin and vinyl butyrate by a factor of 1.5 and 2, respectively. The Km,app for tributyrin was not affected by the Glu87Ala mutation, but the Km,app for vinyl butyrate increased twofold compared to the wild-type lipase. Changing Trp89 into a Phe residue afforded an enzyme with a 2.7- and 2-fold decreased Vmax,app with the substrates tributyrin and vinyl butyrate, respectively, compared to the wild-type lipase. No significant effects on the Km,app values for tributyrin or vinyl butyrate were seen as a result of the Trp89Phe mutation. However, the introduction of a Glu residue at position 89 in the lid increased the Km,app for tributyrin and vinyl butyrate by a factor of > 5 and 2, respectively. The Trp89Glu mutated lipase could not be saturated with tributyrin within the experimental conditions (0-680 mM) studied here. With vinyl butyrate as a substrate the Vmax,app was only 6% of that obtained with wild-type enzyme.

L29 ANSWER 32 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1995:340822 CAPLUS Full-text
DOCUMENT NUMBER: 122:155213
TITLE: Lipase variants, their manufacture with recombinant cells, and their use in detergent

compositions
INVENTOR(S): Svendsen, Allan; Patkar, Shamkant Anant; Gormsen, Erik; Clausen, Ib Groth
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9425577	A1	19941110	WO 1994-DK162	19940422
W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KG, KP, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, TJ, UA, US, UZ, VN				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9465358	A	19941121	AU 1994-65358	19940422
BR 9406384	A	19960116	BR 1994-6384	19940422
EP 695348	A1	19960207	EP 1994-913051	19940422
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
CN 1124039	A	19960605	CN 1994-192175	19940422
JP 08509364	T	19961008	JP 1994-523626	19940422
FI 9505018	A	19951020	FI 1995-5018	19951020

PRIORITY APPLN. INFO.: DK 1993-466 A 19930423
WO 1994-DK162 W 19940422

AB Novel lipase variants with improved properties, DNA constructs coding for the expression of these variants, host cells expressing the lipase variant genes, and methods for preparing the variants by culturing the recombinant cells are claimed. Lipases comprising a trypsin-like catalytic triad including an active serine located in a predominantly hydrophobic, elongated binding pocket of the lipase mol. are mutated so as to substitute a non-aromatic amino acid residue of a lipid contact zone comprising residues located within the part of the lipase structure containing the active serine residue, which residues may participate in the interaction with the substrate at or during hydrolysis, with an aromatic amino acid residue. Several Humicola lanuginosa lipase variants were produced with Aspergillus oryzae and tested for wash performance. The [Trp-96]-, and [Trp-96,Asn-210]- lipase variants exhibited 2.7 and 3.4-fold better wash performance than wild-type lipase.

L29 ANSWER 33 OF 37 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 95115502 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 7815893
TITLE: Trp89 in the lid of Humicola lanuginosa lipase is important for efficient hydrolysis of tributyrin.
AUTHOR: Holmquist M; Martinelle M; Clausen I G; Patkar S; Svendsen A; Hult K
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
SOURCE: Lipids, (1994 Sep) Vol. 29, No. 9, pp. 599-603.
Journal code: 0060450. ISSN: 0024-4201.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 17 Feb 1995
Last Updated on STN: 29 Jan 1999
Entered Medline: 9 Feb 1995

AB To determine whether Trp89 located in the lid of the lipase (EC 3.1.1.3) from Humicola lanuginosa is important for the catalytic property of the enzyme, site-directed mutagenesis at Trp89 was carried out. The kinetic properties of wild type and mutated enzymes were studied with tributyrin as substrate. Lipase variants in which Trp89 was changed to Phe, Leu, Gly or Glu all showed less than 14% of the activity compared to that of the wild type lipase. The Trp89Glu mutant was the least active with only 1% of the activity seen with the wild type enzyme. All Trp mutants had the same binding affinity to the tributyrin substrate interface as did the wild type enzyme. Wild type lipase showed saturation kinetics against tributyrin when activities were measured with mixed emulsions containing different proportions of tributyrin and the nonionic alkyl polyoxyethylene ether surfactant, Triton DF-16. Wild type enzyme showed a Vmax = 6000 +/- 300 mmol.min-1.g-1 and an apparent Km = 16 +/- 2% (vol/vol) for tributyrin in Triton DF-16, while the mutants did not show saturation kinetics in an identical assay. The apparent Km for tributyrin in Triton DF-16 was increased as the result of replacing Trp89 with other residues (Phe, Leu, Gly or Glu). The activities of all mutants were more sensitive to the presence of Triton DF-16 in the tributyrin substrate than was wild type lipase. The activity of the Trp89Glu mutant was decreased to 50% in

the presence of 2 vol% Triton DF-16 compared to the activity seen with pure tributyrin as substrate.(ABSTRACT TRUNCATED AT 250 WORDS)

L29 ANSWER 34 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1995:18786 CAPLUS Full-text
DOCUMENT NUMBER: 122:34002
TITLE: Detergent compositions comprising lipase variants
AUTHOR(S): Novo, Nordisk
CORPORATE SOURCE: Den.
SOURCE: Research Disclosure (1994), 359, 151-6 (No. 35944)
CODEN: RSDSBB; ISSN: 0374-4353
DOCUMENT TYPE: Journal; Patent
LANGUAGE: English
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
RD 359044		19940310		
PRIORITY APPLN. INFO.:			RD 1994-359044	19940310

AB Detergent compns., especially for dishwashing, contain lipase from Humicola lanuginosa for improved effectiveness.

L29 ANSWER 35 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1993:576669 CAPLUS Full-text
DOCUMENT NUMBER: 119:176669
TITLE: Improving the resistance of proteins and enzymes to proteolysis by manipulation of surface structures
INVENTOR(S): Svendsen, Allan; Clausen, Ib Groth; Pathar, Shamkant Anant
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 55 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9311254	A1	19930610	WO 1992-DK351	19921126
W: BR, CA, FI, JP, KR, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
JP 07504807	T	19950601	JP 1992-509724	19921126
EP 667915	A1	19950823	EP 1992-924587	19921126
R: AT, BE, DE, DK, ES, FR, GB, IT, NL				
BR 9206815	A	19951031	BR 1992-6815	19921126
FI 9402467	A	19940526	FI 1994-2467	19940526
PRIORITY APPLN. INFO.:			WO 1991-DK350 A	19911126
			DK 1950-91003 A	19911126
			DK 1950-92003 A	19911126
			WO 1992-DK351 W	19921126

AB Enzymes or other proteins are made more resistant to proteinases by modification of the sequences of surface loops that are common sites of proteolysis. The method is particularly suited for enzymes to be used in proteinase-containing laundry detergents. These sites are identified by very limited proteolysis of the protein and N-terminal sequencing of the cleavage products. Three cleavage sites identified the lipase of Humicola lanuginosa DSM 4106 at positions 162-165, 209-212, and 239-242 were substituted with an array of tetrapeptides by standard methods of site-directed mutagenesis of the cloned gene and the proteins manufactured by expression of the gene in Aspergillus oryzae. Under conditions in which the wild-type proteinase lost 75% of its activity in 90 min upon digestion with Savinase® (subtilisin), one of these variants retained >80% of its activity.

L29 ANSWER 36 OF 37 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 94183411 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 8136025
TITLE: Lipases from Rhizomucor miehei and Humicola lanuginosa: modification of the lid covering the active site alters enantioselectivity.
AUTHOR: Holmquist M; Martinelle M; Berglund P; Clausen I G; Patkar S; Svendsen A; Hult K
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal

Institute of Technology, Stockholm, Sweden.
SOURCE: Journal of protein chemistry, (1993 Dec) Vol. 12, No. 6, pp. 749-57.
Journal code: 8217321. ISSN: 0277-8033.
PUB. COUNTRY: United States
DOCUMENT TYPE: (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 9 May 1994
Last Updated on STN: 29 Jan 1999
Entered Medline: 28 Apr 1994

AB The homologous lipases from Rhizomucor miehei and Humicola lanuginosa showed approximately the same enantioselectivity when 2-methyldecanoic acid esters were used as substrates. Both lipases preferentially hydrolyzed the S-enantiomer of 1-heptyl 2-methyldecanoate (R. miehei: ES = 8.5; H. lanuginosa: ES = 10.5), but the R-enantiomer of phenyl 2-methyldecanoate (ER = 2.9). Chemical arginine specific modification of the R. miehei lipase with 1,2-cyclohexanedione resulted in a decreased enantioselectivity (ER = 2.0), only when the phenyl ester was used as a substrate. In contrast, treatment with phenylglyoxal showed a decreased enantioselectivity (ES = 2.5) only when the heptyl ester was used as a substrate. The presence of guanidine, an arginine side chain analog, decreased the enantioselectivity with the heptyl ester (ES = 1.9) and increased the enantioselectivity with the aromatic ester (ER = 4.4) as substrates. The mutation, Glu 87 Ala, in the lid of the H. lanuginosa lipase, which might decrease the electrostatic stabilization of the open-lid conformation of the lipase, resulted in 47% activity compared to the native lipase, in a tributyrin assay. The Glu 87 Ala mutant showed an increased enantioselectivity with the heptyl ester (ES = 17.4) and a decreased enantioselectivity with the phenyl ester (ER = 2.5) as substrates, compared to native lipase. The enantioselectivities of both lipases in the esterification of 2-methyldecanoic acid with 1-heptanol were unaffected by the lid modifications.

L29 ANSWER 37 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1993:142540 CAPLUS Full-text
DOCUMENT NUMBER: 118:142540
TITLE: Stabilization of enzymes by selective replacement of amino acids with proline
INVENTOR(S): Svendsen, Allan; Von der Osten, Claus; Clausen, Ib Groth; Patkar, Shamkant Anant; Borch, Kim
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9219726	A1	19921112	WO 1992-DK142		19920501
W: FI, JP, KR, US					
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE					
EP 585285	A1	19940309	EP 1992-910123		19920501
EP 585285	B1	19980812			
R: AT, BE, DE, DK, ES, FR, GB, IT, NL, SE					
JP 06507071	T	19940811	JP 1992-509154		19920501
AT 169678	T	19980815	AT 1992-910123		19920501
ES 2121854	T3	19981216	ES 1992-910123		19920501
US 5914306	A	19990622	US 1993-140008		19931022
PRIORITY APPLN. INFO.:			EP 1991-610035	A	19910501
			WO 1992-DK142	W	19920501

AB Enzymes are made more stable by methods that minimize the structural flexibility of the peptide backbone. This is achieved by substituting selected amino acids with proline in regions of the protein outside stable higher order structures such as α-helices and β-sheets. The criteria for selecting amino acids to be substituted also include dihedral angles ψ and ϕ in the ranges -90°<ϕ<-40° and -180°<ψ<-150°, -80<ψ<10, 100°<ψ<180. The invention is intended for use in the preparation of stable enzymes for use in detergents. Genes for a set of derivs. of the lipase of Humicola lanuginosa with individual amino acids substituted by proline were prepared by PCR mutagenesis of the gene and the expression cassette used to manufacture the protein in Aspergillus. An analog of the lipase with Gly225 replaced by Pro lost 50% of its activity after .apprx.30 min at 70° whereas the wild type lost 50% of its activity after 15 min. at the same temperature

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L2            0 FILE CAPLUS
L3            0 FILE SCISEARCH
L4            0 FILE LIFESCI
L5            0 FILE BIOSIS
L6            0 FILE EMBASE

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TOTAL FOR ALL FILES
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=> s l14 not 2000-2007/py
TOTAL FOR ALL FILES
L49          85 L14 NOT 2000-2007/PY

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PROCESSING COMPLETED FOR L49
L50          37 DUP REM L49 (48 DUPLICATES REMOVED)

=> d ibib abs 1-37

L50  ANSWER 1 OF 37  CAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:         1999:231214  CAPLUS  Full-text
DOCUMENT NUMBER:          130:264043
TITLE:                    Lipase variants and their use in
                          detergent compositions
INVENTOR(S):              Svendsen, Allan; Patkar, Shamkant Anant; Gormsen,
                          Erik; Clausen, Ib Groth; Okkels, Jens Sigurd;
                          Thellersen, Marianne
PATENT ASSIGNEE(S):       Novo Nordisk A/S, Den.
SOURCE:                   U.S., 50 pp., Cont.-in-part of U.S. Ser. No. 434,904,
                          abandoned.
                          CODEN: USXXAM
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   5
PATENT INFORMATION:

     PATENT NO.           KIND   DATE        APPLICATION NO.          DATE
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     US 5892013            A     19990406    US 1995-488271           19950905
     CA 2092615            A1    19920314    CA 1991-2092615          19910913
PRIORITY APPLN. INFO.:                       DK 1990-2194         A   19900913
                                             DK 1990-2195         A   19900913
                                             DK 1990-2196         A   19900913
                                             US 1993-977429       B1  19930222
                                             DK 1993-466          A   19930423
                                             DK 1994-217          A   19940222
                                             US 1995-434904       B2  19950501
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AB The present invention relates to lipase variants which exhibit improved properties, detergent compns. comprising said lipase variants, DNA constructs coding for said lipase variants, and methods of making said lipase variants. The variants contain one or more deletions or substitutions of an amino acid residue in the lipid contact zone, or substitutions of two amino

acid residues of the surface loop structure with cysteine which are positioned to form a disulfide bond. Numerous Humicola lanuginosa lipase variants were prepared with recombinant Aspergillus oryzae. Variants R209A+E210A, E56Q, D96L, D96K, D96W, and D96W+E210N had considerably better wash performance than the wild-type lipase. A number of the variants had higher specific activity and/or improved resistance to proteolytic degradation and almost all had improved resistance to alkaline conditions.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 2 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1999:111576 CAPLUS Full-text
DOCUMENT NUMBER: 130:164901
TITLE: Lipase variants and their use in detergent compositions
INVENTOR(S): Svendsen, Allan; Patkar, Shamkant Anant; Gormsen, Erik; Okkels, Jens Sigurd; Thellersen, Marianne
PATENT ASSIGNEE(S): Novo Nordisk A/S, Norway
SOURCE: U.S., 51 pp., Cont.-in-part of U.S. Ser. No. 434,904, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5869438	A	19990209	US 1995-479275	19950607
CA 2092615	A1	19920314	CA 1991-2092615	19910913

PRIORITY APPLN. INFO.:

DK 1990-2194	A	19900913
DK 1990-2195	A	19900913
DK 1990-2196	A	19900913
US 1991-977429	B1	19910913
DK 1993-466	A	19930423
DK 1994-217	A	19940222
US 1995-434904	B2	19950501

AB The present invention relates to lipase variants which exhibit improved properties and to detergent additives and detergent compns. comprising said lipase variants. The variants contain one or more deletions or substitutions of an amino acid residue in the lipid contact zone, or substitutions of two amino acid residues of the surface loop structure with cysteine which are positioned to form a disulfide bond. Numerous Humicola lanuginosa lipase variants were prepared with recombinant Aspergillus oryzae. Variants R209A+E210A, E56Q, D96L, D96K, D96W, and D96W+E210N had considerably better wash performance than the wild-type lipase. A number of the variants had higher specific activity and/or improved resistance to proteolytic degradation and almost all had improved resistance to alkaline conditions.

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 3 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1999:785630 SCISEARCH Full-text
THE GENUINE ARTICLE: 246MN
TITLE: Computational analysis of chain flexibility and fluctuations in Rhizomucor miehei lipase
AUTHOR: Peters G H; Bywater R P (Reprint)
CORPORATE SOURCE: Novo Nordisk AS, Biostruct Grp, DK-2760 Malov, Denmark (Reprint); Tech Univ Denmark, Dept Chem, DK-2800 Lyngby, Denmark
COUNTRY OF AUTHOR: Denmark
SOURCE: PROTEIN ENGINEERING, (SEP 1999) Vol. 12, No. 9, pp. 747-754.
ISSN: 0269-2139.
PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 77
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have performed molecular dynamics simulation of Rhizomucor miehei lipase (Rml) with explicit water molecules present. The simulation was carried out in periodic boundary conditions and conducted for 1.2 ns in order to determine the concerted protein dynamics and to examine how well the essential motions are preserved along the trajectory. Protein motions are extracted by means of the essential dynamics analysis method for different

lengths of the trajectory. Motions described by eigenvector 1 converge after approximately 200 ps and only small changes are observed with increasing simulation time. Protein dynamics along eigenvectors with larger indices, however, change with simulation time and generally, with increasing eigenvector index, longer simulation times are required for observing similar protein motions (along a particular eigenvector). Several regions in the protein show relatively large fluctuations and in particular motions in the active site lid and the segments Thr57-Asn63 and the active site hinge region Pro101-Gly104 are seen along several eigenvectors. These motions are generally associated with glycine residues, while no direct correlations are observed between these fluctuations and the positioning of prolines in the protein structure. The partial opening/closing of the lid is an example of induced fit mechanisms seen in other enzymes and could be a general mechanism for the activation of Rml.

L50 ANSWER 4 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1999:50858 SCISEARCH Full-text
THE GENUINE ARTICLE: 155QB
TITLE: Stereoselectivity of Mucorales lipases toward triradylglycerols - A simple solution to a complex problem
AUTHOR: Scheib H; Pleiss J; Kovac A; Paltauf F; Schmid R D (Reprint)
CORPORATE SOURCE: Univ Stuttgart, Inst Tech Biochem, Allmandring 31, D-70569 Stuttgart, Germany (Reprint); Univ Stuttgart, Inst Tech Biochem, D-70569 Stuttgart, Germany; Graz Tech Univ, Dept Biochem & Food Chem, A-8010 Graz, Austria
COUNTRY OF AUTHOR: Germany; Austria
SOURCE: PROTEIN SCIENCE, (JAN 1999) Vol. 8, No. 1, pp. 215-221. ISSN: 0961-8368.
PUBLISHER: COLD SPRING HARBOR LAB PRESS, 1 BUNGTOWN RD, PLAINVIEW, NY 11724 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 21
ENTRY DATE: Entered STN: 1999
Last Updated on STN: 1999
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The lipases from Rhizopus and Rhizomucor are members of the family of Mucorales lipases. Although they display high sequence homology, their stereoselectivity toward triradylgrycerols (sn-2 substituted triacylglycerols) varies. Four different triradylglycerols were investigated, which were classified into two groups: flexible substrates with rotatable O'-C1' ether or ester bonds adjacent to C2 of glycerol and rigid substrates with a rigid N'-C1' amide bond or a phenyl ring in sn-2. Although Rhizopus lipase shows opposite stereopreference for flexible and rigid substrates (hydrolysis in sn-1 and sn-3, respectively), Rhizomucor lipase hydrolyzes both groups of triradylglycerols preferably in sn-1. To explain these experimental observations, computer-aided molecular modeling was applied to study the molecular basis of stereoselectivity. A generalized model for both lipases of the Mucorales family highlights the residues mediating stereoselectivity: (1) L258, the C-terminal neighbor of the catalytic histidine, and (2) G266, which is located in a loop contacting the glycerol backbone of a bound substrate. Interactions with triradylglycerol substrates are dominated by van der Waals contacts. Stereoselectivity can be predicted by analyzing the value of a single substrate torsion angle that discriminates between sn-1 and sn-3 stereopreference for all substrates and lipases investigated here. This simple model can be easily applied in enzyme and substrate engineering to predict Mucorales lipase variants and synthetic substrates with desired stereoselectivity.

L50 ANSWER 5 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1999:287580 CAPLUS Full-text
DOCUMENT NUMBER: 131:84811
TITLE: Steady state fluorescence studies on the interaction of Humicola lanuginosa lipase with lipid vesicles
AUTHOR(S): Cajal, Y.; Prat, J.; Svendsen, A.; De Bolos, J.; Alsina, M. A.
CORPORATE SOURCE: Department of Physical Chemistry, School of Pharmacy, University of Barcelona, Barcelona, 08028, Spain
SOURCE: Biomedical Chromatography (1999), 13(2), 157-158
CODEN: BICHE2; ISSN: 0269-3879
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In this work, the conformational changes that take place in Humicola lanuginosa lipase (HLL) upon binding to vesicles of 1-palmitoyl-2-oleoylglycero-sn-phosphoglycerol (POPG), and also in the

presence of tributyrin (TB) as substrate, were studied by fluorescence techniques. The catalytic activity of the enzyme under the same conditions as used for fluorescence was measured spectroscopically by UV using p-nitrophenylbutyrate as substrate. An inactive mutant, with active site Ser-146 mutated to Ala, was used in the fluorescence expts. to avoid interference from product formation.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 6 OF 37 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 1998400942 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 9730809
TITLE: Active serine involved in the stabilization of the active site loop in the Humicola lanuginosa lipase.
AUTHOR: Peters G H; Svendsen A; Langberg H; Vind J; Patkar S A; Toxvaerd S; Kinnunen P K
CORPORATE SOURCE: Chemistry Department III, H.C. Orsted Institutet, University of Copenhagen, Denmark.. ghp@st.ki.ku.dk
SOURCE: Biochemistry, (1998 Sep 8) Vol. 37, No. 36, pp. 12375-83. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 23 Feb 1999
Last Updated on STN: 23 Feb 1999
Entered Medline: 5 Feb 1999

AB We have investigated the binding properties of and dynamics in Humicola lanuginosa lipase (Hll) and the inactive mutant S146A (active Ser146 substituted with Ala) using fluorescence spectroscopy and molecular dynamics simulations, respectively. Hll and S146A show significantly different binding behavior for phosphatidylcholine (PC) and phosphatidylglycerol (PG) liposomes. Generally, higher binding affinity is observed for Hll than the S146A mutant. Furthermore, depending on the matrix, the addition of the transition state analogue benzene boronic acid increases the binding affinity of S146A, whereas only small changes are observed for Hll suggesting that the active site lid in the latter opens more easily and hence more lipase molecules are bound to the liposomes. These observations are in agreement with molecular dynamics simulations and subsequent essential dynamics analyses. The results reveal that the hinges of the active site lid are more flexible in the wild-type Hll than in S146A. In contrast, larger fluctuations are observed in the middle region of the active site loop in S146A than in Hll. These findings reveal that the single mutation (S146A) of the active site serine leads to substantial conformational alterations in the H. lanuginosa lipase and different binding affinities.

L50 ANSWER 7 OF 37 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 1998244839 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 9578545
TITLE: Interfacial activation of triglyceride lipase from Thermomyces (Humicola) lanuginosa: kinetic parameters and a basis for control of the lid.
AUTHOR: Berg O G; Cajal Y; Butterfoss G L; Grey R L; Alsina M A; Yu B Z; Jain M K
CORPORATE SOURCE: Department of Chemistry and Biochemistry, University of Delaware, Newark 19716, USA.
CONTRACT NUMBER: GM29703 (NIGMS)
SOURCE: Biochemistry, (1998 May 12) Vol. 37, No. 19, pp. 6615-27. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: (COMPARATIVE STUDY) Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T) (RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 11 Jun 1998
Last Updated on STN: 29 Jan 1999
Entered Medline: 4 Jun 1998

AB A strategy is developed to analyze steady-state kinetics for the hydrolysis of a soluble substrate partitioned into the interface by an enzyme at the interface. The feasibility of this approach to obtain interfacial primary kinetic and equilibrium parameters is demonstrated for a triglyceride lipase. Analysis for phospholipase A2 catalyzed hydrolysis of rapidly exchanging micellar (Berg et al. (1997) Biochemistry 36, 14512-14530) and nonexchangeable vesicular (Berg et al., (1991) Biochemistry 30, 7283-7291) phospholipids is extended to include the case of a substrate that does

not form the interface. The triglyceride lipase (tlTGL) from Thermomyces (formerly Humicola) lanuginosa hydrolyzes p-nitrophenylbutyrate or tributyrin partitioned in the interface of 1-palmitoyl-2-oleoyl-sn-glycero-3- phosphoglycerol (POPG) vesicles at a rate that is more than 100-fold higher than that for the monodispersed substrate or for the substrate partitioned into zwitterionic vesicles. Catalysis and activation is not seen with the S146A mutant without the catalytic serine-146; however, it binds to the POPG interface with the same affinity as the WT. Thus POPG acts as a diluent surface to which the lipase binds in an active, or "open", form for the catalytic turnover; however, the diluent molecules have poor affinity for the active site. Analysis of the substrate and the diluent concentration dependence of the rate of hydrolysis provides a basis for the determination of the primary interfacial catalytic parameters. As a competitive substrate, tributyrin provided a check for the apparent affinity parameters. Nonidealities from the fractional difference in the molecular areas in interfaces are expressed as the area correction factor and can be interpreted as a first-order approximation for the interfacial activity coefficient. The basis for the interfacial activation of tlTGL on anionic interface is attributed to cationic R81, R84, and K98 in the "hinge" around the 86-93 "lid" segment of tlTGL.

L50 ANSWER 8 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 1998:711158 CAPLUS Full-text
DOCUMENT NUMBER: 130:92064
TITLE: Spectroscopic studies of the interfacial binding of Humicola lanuginosa lipase
AUTHOR(S): Cajal, Yolanda; Prat, Josefina; De Bolos, Jordi; Asuncion Alsina, M.; Svendsen, Allan
CORPORATE SOURCE: School of Pharmacy, Department of Physical Chemistry, University of Barcelona, Barcelona, 08028, Spain
SOURCE: Analyst (Cambridge, United Kingdom) (1998), 123(11), 2229-2233
CODEN: ANALAO; ISSN: 0003-2654
PUBLISHER: Royal Society of Chemistry
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The interaction of Humicola lanuginosa lipase (HLL) with small unilamellar vesicles of 1-palmitoyl-2-oleoylglycero-sn-3- phosphoglycerol (POPG) and in the presence of tributyrin (TB) as a substrate was studied by the use of steady-state fluorescence techniques. An inactive mutant with the serine from the catalytic triad changed by alanine (S146A) was used in expts. with TB to avoid interferences from product formation. HLL binds to POPG vesicles in an active or open form for the catalytic turnover, therefore POPG provides a suitable system for studying the conformational changes involving the movement of the loop of amino acids that covers the active site of the enzyme in solution Tryptophan (Trp) fluorescence shows that HLL binding to POPG occurs with a change in the environment of Trp residue(s) and that there is only one type of bound form, even in the presence of TB. Accessibility to aqueous quenchers indicates shielding of Trp in the membrane. Fluorescence anisotropy of the enzyme increases on binding to the vesicles, indicating restricted rotational freedom for the Trp due to penetration in the bilayer. Resonance energy transfer expts. using an interfacial membrane probe, 1-[4-(trimethylammonio)phenyl]-6-phenylhexa- 1,3,5-triene p-toluenesulfonate (TMA-DPH), and an internal membrane probe, 1,6-diphenylhexa-1,3,5-triene (DPH), indicate that HLL does not penetrate very deeply in the hydrophobic core of the membrane, but preferentially stays close to the lipid interface. Addition of substrate (TB) does not result in any addnl. changes in the spectroscopic properties of HLL. It is suggested that the observed changes are due to the 'opening of the lid' on binding to POPG vesicles, leaving the active site accessible for the substrate to bind.
REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 9 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:229525 SCISEARCH Full-text
THE GENUINE ARTICLE: ZC005
TITLE: A proposed architecture for lecithin cholesterol acyl transferase (LCAT): Identification of the catalytic triad and molecular modeling
AUTHOR: Peelman F; Vinaimont N; Verhee A; Vanloo B; Verschelde J L; Labeur C; Seguret-Mace S; Duverger N; Hutchinson G; Vandekerckhove J; Tavernier J; Rosseneu M (Reprint)
CORPORATE SOURCE: Univ Ghent, Dept Biochem, Lab Lipoprot Chem, Hosp Str 13, B-9000 Ghent, Belgium (Reprint); Univ Ghent, Dept Biochem, Lab Lipoprot Chem, B-9000 Ghent, Belgium; State Univ Ghent VIB, Fac Med, Dept Biochem, B-9000 Ghent, Belgium; Rhone Poulenc Rorer, GENCELL, Cardiovasc Dept, Vitry Sur Seine, France; Univ Coll London, Dept Biochem, London, England
maryvonne.rosseneu@rug.ac.be
COUNTRY OF AUTHOR: Belgium; France; England
SOURCE: PROTEIN SCIENCE, (MAR 1998) Vol. 7, No. 3, pp. 587-599.
ISSN: 0961-8368.

PUBLISHER: COLD SPRING HARBOR LAB PRESS, PUBLICATIONS DEPT, 500 SUNNYSIDE BLVD, WOODBURY, NY 11797-2924 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 52
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 15 Mar 2007
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The enzyme cholesterol lecithin acyl transferase (LCAT) shares the Ser/Asp-Glu/His triad with lipases, esterases and proteases, but the low level of sequence homology between LCAT and these enzymes did not allow for the LCAT fold to be identified yet. We, therefore, relied upon structural homology calculations using threading methods based on alignment of the sequence against a library of solved three-dimensional protein structures, for prediction of the LCAT fold. We propose that LCAT, like lipases, belongs to the alpha/beta hydrolase fold family, and that the central domain of LCAT consists of seven conserved parallel beta-strands connected by four alpha-helices and separated by loops. We used the conserved features of this protein fold for the prediction of functional domains in LCAT, and carried out site-directed mutagenesis for the localization of the active site residues. The wild-type enzyme and mutants were expressed in Cos-1 cells. LCAT mass was measured by ELISA, and enzymatic activity was measured on recombinant HDL, on LDL and on a monomeric substrate. We identified D345 and H377 as the catalytic residues of LCAT, together with F103 and L182 as the oxyanion hole residues. In analogy with lipases, we further propose that a potential "lid" domain at residues 50-74 of LCAT might be involved in the enzyme-substrate interaction. Molecular modeling of human LCAT was carried out using human pancreatic and Candida antarctica lipases as templates. The three-dimensional model proposed here is compatible with the position of natural mutants for either LCAT deficiency or Fish-eye disease. It enables moreover prediction of the LCAT domains involved in the interaction with the phospholipid and cholesterol substrates.

L50 ANSWER 10 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 1998:811251 CAPLUS Full-text
DOCUMENT NUMBER: 130:164804
TITLE: Fluorescence study of fungal lipase from Humicola lanuginosa
AUTHOR(S): Stobiecka, A.; Wysocki, S.; Brzozowski, A. M.
CORPORATE SOURCE: Inst. General Food Chem., Tech. Univ., Lodz, 90-924, Pol.
SOURCE: Journal of Photochemistry and Photobiology, B: Biology (1998), 45(2-3), 95-102
CODEN: JPPBEG; ISSN: 1011-1344
PUBLISHER: Elsevier Science S.A.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Time-resolved and steady-state fluorescence quenching measurements have been performed to study two different conformations of the fungal lipase from Humicola lanuginosa. The intrinsic fluorescence of tryptophan Trp89 residue, located in the 'lid' region, has been used as a probe for the dynamics of protein. The native ('closed-lid') form of the enzyme has been found to decay as a triple exponential with time consts. and relative contributions of 5.4 ns (74.3%), 2.2 ns (20.4%) and 0.4 ns (5.3%). A comparison of recovered decay parameters obtained for native and mutated H. lanuginosa lipase shows that Trp89 contributes about 61% to the class of emitting species with the lifetime of 5.4 ns. The fluorescence quenching data show that three out of four tryptophans (i.e., 117, 221 and 260 residues) within H. lanuginosa lipase are totally quenchable by acrylamide while completely inaccessible to iodide. On the contrary, the Trp89 residue is available for both quenchers. Using steady-state iodide fluorescence quenching data and the fluorescence-quenching-resolved-spectra (FQRS) method, the total emission spectrum of the native lipase has been decomposed into two spectral components. One of them, unquenchable by iodide, has a maximum of fluorescence emission at 330 nm and the second one, exposed to the solvent, emits at 338 nm. The resolved spectrum of the redder component corresponds to the Trp89 residue, which participates in about 65% of the total H. lanuginosa emission. The dynamic Stern-Volmer quenching consts. calculated for both native ('closed-lid') and inhibited ('open-lid') lipase are 2.71 and 4.49 M-1, resp. The values obtained indicate that Trp89 is not deeply buried in the protein matrix. Our results suggest that distinct configurations of fungal lipase can be monitored using the fluorescence of the Trp89 residue located in the 'lid'-helix which participates in an interfacial activation of the enzyme.
REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 11 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:587613 SCISEARCH Full-text
THE GENUINE ARTICLE: 106NT
TITLE: Anatomy of lipase binding sites: the scissile fatty acid binding site
AUTHOR: Pleiss J; Fischer M; Schmid R D (Reprint)

CORPORATE SOURCE: Univ Stuttgart, Inst Tech Biochem, Allmandring 31, D-70569 Stuttgart, Germany (Reprint); Univ Stuttgart, Inst Tech Biochem, D-70569 Stuttgart, Germany
COUNTRY OF AUTHOR: Germany
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No. 1-2, pp. 67-80.
ISSN: 0009-3084.
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 41
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Shape and physico-chemical properties of the scissile fatty acid binding sites of six lipases and two serine esterases were analyzed and compared in order to understand the molecular basis of substrate specificity. All eight serine esterases and lipases have similar architecture and catalytic mechanism of ester hydrolysis, but different substrate specificities for the acyl moiety. Lipases and esterases differ in the geometry of their binding sites, lipases have a large, hydrophobic scissile fatty acid binding site, esterases like acetylcholinesterase and bromoperoxidase have a small acyl binding pocket, which fits exactly to their favorite substrates. The lipases were subdivided into three sub-groups: (1) lipases with a hydrophobic, crevice-like binding site located near the protein surface (lipases from Rhizomucor and Rhizopus); (2) lipases with a funnel-like binding site (lipases from Candida antarctica, Pseudomonas and mammalian pancreas and cutinase); and (3) lipases with a tunnel-like binding site (lipase from Candida rugosa). The length of the scissile fatty acid binding site varies considerably among the lipases between 7.8 Angstrom in cutinase and 22 Angstrom in Candida rugosa and Rhizomucor miehei lipase. Location and properties of the scissile fatty acid binding sites of all lipases of known structure were characterized. Our model also identifies the residues which mediate chain length specificity and thus may guide protein engineering of lipases for changed chain length specificity. The model was supported by published experimental data on the chain length specificity profile of various lipases and on mutants of fungal lipases with changed fatty acid chain length specificity. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.

L50 ANSWER 12 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:587612 SCISEARCH Full-text
THE GENUINE ARTICLE: 106NT
TITLE: Insights into the molecular basis for fatty acyl specificities of lipases from Geotrichum candidum and Candida rugosa
AUTHOR: Holmquist M (Reprint)
CORPORATE SOURCE: Royal Inst Technol, Dept Biochem & Biotechnol, SE-10044 Stockholm, Sweden (Reprint)
COUNTRY OF AUTHOR: Sweden
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No. 1-2, pp. 57-66.
ISSN: 0009-3084.
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 49
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Despite immense progress in our comprehension of lipase structure and function during the past decade, the basis for lipase acyl specificities has remained poorly understood. This review summarizes some recent advances in the understanding at the molecular-level of substrate acyl recognition by two members in a group of large (M-w similar to 60 kDa) microbial lipases. Two aspects of acyl specificity will be focused upon. (1) The unique preference of a fungal Geotrichum candidum lipase for long-chain cis (Delta-9) unsaturated fatty acid moieties in the substrate. Mutational analysis of this lipase identified residues essential for its anomalous acyl preference. This information highlighted for the first time parts in the lipase molecule involved in substrate acyl differentiation. These results are discussed in the context of the 3D-structure of a G. candidum lipase isoenzyme and structures of the related Candida rugosa lipase in complex with inhibitors. (ii) The mechanism by which the yeast C. rugosa lipase discriminates between enantiomers of a substrate with a chiral acyl moiety. Molecular modeling in combination with substrate engineering and kinetic analyses, identified two alternative substrate binding modes. This allowed for the proposal of a molecular mechanism explaining how long-chain alcohols can

act as enantioselective inhibitors of this enzyme. A picture is thus beginning to emerge of the interplay between lipase structure and fatty acyl specificity. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.

L50 ANSWER 13 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 5
ACCESSION NUMBER: 1998:455633 CAPLUS Full-text
DOCUMENT NUMBER: 129:213493
TITLE: The decay of the fluorescence anisotropy of tryptophan residues in fungal lipase from Humicola lanuginosa
AUTHOR(S): Stobiecka, A.; Wysocki, S.
CORPORATE SOURCE: Institute General Food Chemistry, Technical University Lodz, Lodz, 90924, Pol.
SOURCE: Journal of Radioanalytical and Nuclear Chemistry (1998), 232(1-2), 43-48
CODEN: JRNCDM; ISSN: 0236-5731
PUBLISHER: Elsevier Science S.A.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Pulse nanosecond fluorescence anisotropy decay was used to study the mobility of Trp residues within fungal lipase from Humicola lanuginosa. The decay of emission anisotropy of protein in native, inhibited, and mutated form was investigated in buffered water and 50% volume/volume glycerol solns. The rotational motions of the lipase were analyzed in terms of 2 different kinetic models. The fluorescence emission anisotropy decay can best be described with 2 rotational correlation times : 0.63 and 5.45 ns in water and 0.98 and 10.70 ns and in 50% volume/volume glycerol solution The decay of inhibited and mutated H. lanuginosa lipase showed a similar biexponential character. These results are interpreted in terms of local or segmental motion arising from a mass of about 1083 Da which corresponds to the 'lid'-helix fragment of the enzyme.

L50 ANSWER 14 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:587609 SCISEARCH Full-text
THE GENUINE ARTICLE: 106NT
TITLE: Biochemical properties of staphylococcal (phospho) lipases
AUTHOR: Simons J W F A; Gotz F; Egmond M R; Verheij H M (Reprint)
CORPORATE SOURCE: Univ Utrecht, Inst Biomembranes, Ctr biomembranes & Lipid Enzymol, Dept Enzymol & Prot Engn, POB 80054, Padualaan 8, NL-3508 TB Utrecht, Netherlands (Reprint); Univ Utrecht, Inst Biomembranes, Ctr biomembranes & Lipid Enzymol, Dept Enzymol & Prot Engn, NL-3508 TB Utrecht, Netherlands; Univ Tubingen, Tubingen, Germany
COUNTRY OF AUTHOR: Netherlands; Germany
SOURCE: CHEMISTRY AND PHYSICS OF LIPIDS, (JUN 1998) Vol. 93, No. 1-2, pp. 27-37.
ISSN: 0009-3084.
PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 37
ENTRY DATE: Entered STN: 1998
Last Updated on STN: 1998
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Various staphylococci secrete lipases which require calcium ions for activity, and have profound preferences for substrates with different chain lengths. The lipase from Staphylococcus hyicus is exceptional since it has higher phospholipase than lipase activity. This paper gives an overview of the biochemical properties: of these enzymes. It appears that chain length selectivity of these enzymes resides in the acylation step. Interfaces mainly influence the acylation step. Calcium ions do not influence the rate of acylation or deacylation although stabilise the enzyme against denaturation. Molecular modelling based on the X-ray structure of Pseudomonas glumae lipase was used to construct a model of the staphylococcal lipases. With this model the position of serveral residues involved in stubstrate selectivity was predicted. Moreover, a sequence element could be assigned that may function as the so-called lid domain in staphylococcal lipases. Sequence alignment of four staphylococcal lipases, and lipases from P. glumae and Bacillus thermocatenulatus identified several potential calcium ligands, one of which was verified by site directed mutagensesis. It is concluded that stabilisation of lipases by calcium ions might be a more general phenomenon than recognized so far. (C) 1998 Elsevier Science Ireland Ltd. All rights reserved.

L50 ANSWER 15 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:757085 CAPLUS Full-text
DOCUMENT NUMBER: 128:24317
TITLE: Detergent compositions containing a specific lipolytic enzyme and alkyl polyglycoside surfactant
INVENTOR(S): Baeck, Andre Cesar; Kasturi, Chandrika
PATENT ASSIGNEE(S): Procter and Gamble Company, USA; Baeck, Andre Cesar; Kasturi, Chandrika
SOURCE: PCT Int. Appl., 74 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9743377	A1	19971120	WO 1996-US7088	19960515
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9658618	A	19971205	AU 1996-58618	19960515
EP 912683	A1	19990506	EP 1996-920251	19960515
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
BR 9612608	A	19990720	BR 1996-12608	19960515
PRIORITY APPLN. INFO.:			WO 1996-US7088 W	19960515

AB The present invention relates to detergent compns. containing a variants of the native lipolytic enzyme producible by Humicola lanuginosa and Thermomyces lanuginosus or by cloning and expressing the gene responsible for producing the variants into a host organism and an ROGx surfactant (R = C10-16 alkyl, G = reducing saccharide containing 5-6 C atoms, x = 1-3). Such compns. provide improved overall detergency performance: reduced redeposition of greasy/oily substances on fabrics, dishware and hard surfaces thereby improving whiteness maintenance, cleaning, spotting, filming and stain removal performances.

L50 ANSWER 16 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:757082 CAPLUS Full-text
DOCUMENT NUMBER: 128:36417
TITLE: Detergent compositions comprising specific lipolytic enzyme and a soil release polymer
INVENTOR(S): Baeck, Andre Cesar; Kasturi, Chandrika
PATENT ASSIGNEE(S): Procter and Gamble Company, USA; Baeck, Andre Cesar; Kasturi, Chandrika
SOURCE: PCT Int. Appl., 77 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9743374	A1	19971120	WO 1996-US7085	19960515
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2254919	A1	19971120	CA 1996-2254919	19960515
AU 9657957	A	19971205	AU 1996-57957	19960515
BR 9612614	A	19990720	BR 1996-12614	19960515
EP 931134	A1	19990728	EP 1996-914666	19960515
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
PRIORITY APPLN. INFO.:			WO 1996-US7085 A	19960515

AB Lipolytic enzymes are variants of the native lipolytic enzymes producible by Humicola lanuginosa and Thermomyces lanuginosus. A soil release polymer is selected from sulfonated poly(ethoxy)/(propoxy)-end capped ester oligomers and/or short block polymers manufactured from di-Me terephthalate (I), 1,2-propylene glycol (II), Me-capped polyethylene glycol and/or short block polymers manufactured from I, II, and sulfoethoxylates. Thus, a granular detergent contained a Na linear C12-alkyl benzenesulfonate 22, a phosphate 23, a carbonate 23, a silicate 14, Zeolite A 8.2, Dequest 2060 0.4, Na sulfate 5.5, amylase 0.005, protease 0.01, pectinase 0.02, Lipolase Ultra 0.005, a soil release polymer 0.2, cellulase 0.001, and water and minors to 100%.

L50 ANSWER 17 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:220628 CAPLUS Full-text
DOCUMENT NUMBER: 126:208956
TITLE: Recombinant lipases with C- and/or N-terminal extensions and their use in detergents
INVENTOR(S): Fuglsang, Claus Crone; Okkels, Jens Sigurd; Pertersen, Dorte Aaby; Patkar, Shamkant Anant; Thellersen, Marianne; Vind, Jesper; Halkier, Torben; Joergensen, Steen Troels; et al.
PATENT ASSIGNEE(S): Novo Nordisk A/s, Den.; Fuglsang, Claus Crone; Okkels, Jens Sigurd; Pertersen, Dorte Aaby; Patkar, Shamkant Anant; Thellersen, Marianne; Vind, Jesper; Halkier, Torben
SOURCE: PCT Int. Appl., 197 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9704078	A1	19970206	WO 1996-DK321	19960712
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA				
AU 9664140	A	19970218	AU 1996-64140	19960712
CN 1193346	A	19980916	CN 1996-196371	19960712
PRIORITY APPLN. INFO.:			DK 1995-832 A	19950714
			DK 1995-1013 A	19950913
			DK 1995-1096 A	19950929
			DK 1995-1306 A	19951121
			US 1996-11634P P	19960214
			DK 1996-372 A	19960401
			US 1996-20461P P	19960507
			WO 1996-DK321 W	19960712

AB The invention relates to a modified enzyme with lipolytic activity recovered from a filamentous fungi or a bacteria having one or more peptide addns. at the N-terminal and/or the C-terminal ends in comparison to the parent enzyme. The peptide addns. significantly improve the washing performance of the lipase. Further, the invention relates to a DNA sequence encoding said modified enzyme, a vector comprising said DNA sequence, a host cell harboring said DNA sequence or said vector, and a process for producing said modified enzyme with lipolytic activity. The lipase variants are useful in detergent compns. Numerous lipase variants containing substitution mutations and C- and/or N-terminal addns. were prepared with recombinant Aspergillus oryzae or with Escherichia coli. The addition of SPIRR to the N-terminus of Humicola lanuginosa lipase increased the wash performance relative to the parent enzyme twofold.

L50 ANSWER 18 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1997:897558 SCISEARCH Full-text
THE GENUINE ARTICLE: YJ709
TITLE: Identification of residues essential for differential fatty acyl specificity of Geotrichum candidum lipases I and II
AUTHOR: Holmquist M (Reprint); Tessier D C; Cygler M
CORPORATE SOURCE: NATL RES COUNCIL CANADA, BIOTECHNOL RES INST, MONTREAL, PQ H4P 2R2, CANADA; ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, S-10044 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: CANADA; SWEDEN
SOURCE: BIOCHEMISTRY, (2 DEC 1997) Vol. 36, No. 48, pp. 15019-15025.

ISSN: 0006-2960.
PUBLISHER: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 44
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The fungus Geotrichum candidum produces two lipase isoenzymes, GCL I and GCL II, with distinct differences in substrate specificity despite their 86% identical primary structure, GCL I prefers ester substrates with long-chain cis (Delta-9) unsaturated fatty acid moieties, whereas GCL II also accepts medium-length (C8-C14) acyl moieties in the substrate. To reveal structural elements responsible for differences in substrate differentiating ability of these isoenzymes, we designed, expressed, and characterized 12 recombinant lipase variants. Three chimeric lipases containing unique portions of the N-terminal and the C-terminal part of GCL I and GCL II, respectively, were constructed and enzymatically characterized. Activities were measured against mixed triglyceride-poly(dimethyl siloxane) particles. Our results indicate that residues within sequence positions 349-406 are essential for GCL I's high triolein/trioctanoin activity ratio of 20. The substitution of that segment in the specific GCL I to the corresponding residues in the nonspecific GCL II, resulted in an enzyme with a triolein/trioctanoin activity ratio of 1.4, identical to that of GCL IL. The reverse mutation in GCL II increased its specificity for triolein by a factor of 2, thus only in part restoring the high specificity seen with GCL I. In further experiments, the point mutations at the active site entrance of the GCL I, Leu358Phe and Ile357Ala/Leu358Phe, lowered the triolein/trioctanoin activity ratio from 20 to 4 and 2.5, respectively. The substitutions Cys379Phe/Ser380Tyr at the bottom of the active site cavity of GCL I decreased its specificity to a value of 3.6, Measurements of lipase activity with substrate particles composed of pure triglycerides or ethyl esters of oleic and octanoic acids resulted in qualitatively similar results as reported above. Our data reveal for the first time the identity of residues essential for the unusual substrate preference of GCL I and show that the anatomy, both at the entrance and the bottom of the active site cavity, plays a key role in substrate discrimination.

L50 ANSWER 19 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 1997:372120 CAPLUS Full-text
DOCUMENT NUMBER: 126:327220
TITLE: Comparison of the adsorption and activity of lipases from Humicola lanuginosa and Candida antarctica on solid surfaces
AUTHOR(S): Wannerberger, Kristin; Arnebrant, Thomas
CORPORATE SOURCE: Department of Food Technology, University of Lund, Lund, S-221 00, Swed.
SOURCE: Langmuir (1997), 13(13), 3488-3493
CODEN: LANGD5; ISSN: 0743-7463
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The adsorption of lipases from H. lanuginosa, the wild type (WT) and a mutant with increased hydrophobicity in the active site region, and lipase B from C. antarctica to solid surfaces was studied by in situ ellipsometry. In addition, the activity of the adsorbed lipase was measured in situ and from the different surface concns., the specific activity was calculated Concentration- and temperature-dependence as well as the influence of surface wettability was studied using silica surfaces with varying degrees of methylation. The higher hydrophobicity of the mutant compared to the WT resulted in increased amts. adsorbed, no desorbable fraction during rinsing, and absence of an initial maximum in adsorbed amount (as seen for the WT) at higher concns. No temperature dependence for the mutant could be observed This was in contrast to the WT where both the plateau value of the adsorbed amount and the activity decreased with increasing temperature The influence of surface wettability was similar for both featuring a decreased adsorbed amount and increased specific activity with increasing wettability. The amount adsorbed of the Candida lipase was significantly higher at all concns., compared to the other lipases, and the activity was very low, indicating adsorption with the active site region directed toward the surface. The surface wettability did not affect the activity of Candida lipase.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L50 ANSWER 20 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1997:548646 CAPLUS Full-text
DOCUMENT NUMBER: 127:189671
TITLE: Biotechnology in detergents and cosmetics
AUTHOR(S): Ito, Susumu
CORPORATE SOURCE: Tochigi Res. Lab., Kao Corp., Tochigi, 321-34, Japan
SOURCE: Baiosaiensu to Indasutori (1997), 55(8), 541-545
CODEN: BIDSE6; ISSN: 0914-8981

PUBLISHER: Baioindasutori Kyokai
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review with 18 refs. on site-directed mutation of subtilisin suitable for detergents, heat-stable mutation of Bacillus licheniformis amylase, amylopullulanase, site-directed mutation of α-amylase to improve durability against chelating agents and heat, alkaline cellulase of alkalophilic Bacillus, cellulase of Aspergillus oryzae, production of lipase of Humicola lanuginosa by A. oryzae as host organism, catalytic site of Mocor miehei enzyme, raw materials for cosmetics, sophorolipid, kojic acid, hyaluronate, γ-linolenate, shikonin, and tuberose polysaccharide.

L50 ANSWER 21 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1997:250761 SCISEARCH Full-text
THE GENUINE ARTICLE: WP714
TITLE: Computational studies of the activation of lipases and the effect of a hydrophobic environment
AUTHOR: Peters G H (Reprint); Toxvaerd S; Olsen O H; Svendsen A
CORPORATE SOURCE: UNIV COPENHAGEN, HC ORSTED INST, DEPT CHEM 3, UNIV PK 5, DK-2100 COPENHAGEN O, DENMARK (Reprint); NOVO NORDISK AS, DK-2880 BAGSVAERD, DENMARK
COUNTRY OF AUTHOR: DENMARK
SOURCE: PROTEIN ENGINEERING, (FEB 1997) Vol. 10, No. 2, pp. 137-147.
ISSN: 0269-2139.
PUBLISHER: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD, ENGLAND OX2 6DP.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 69
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB We have investigated the activation pathway of three wild type lipases and three mutants using molecular dynamics techniques combined with a constrained mechanical protocol. The activation of these lipases involves a rigid body hinge-type motion of a single helix, which is displaced during activation to expose the active site and give access to the substrate. Our results suggest that the activation of lipases is enhanced in a hydrophobic environment as is generally observed in experiments. The energy gain upon activation varies between the different lipases and depends strongly on the distribution of the charged residues in the activating loop region. In a low dielectric constant medium (such as a lipid environment), the electrostatic interactions between the residues located in the vicinity of the activating loop (lipid contact zone) are dominant and determine the activation of the lipases. Calculations of the pK(a)s qualitatively indicate that some titratable residues experience significant pK shifts upon activation. These calculations may provide sufficient details for an understanding of the origin and magnitude of a given electrostatic effect and may provide an avenue for exploring the activation pathway of lipases.

L50 ANSWER 22 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1997:170960 SCISEARCH Full-text
THE GENUINE ARTICLE: WJ876
TITLE: Altered acyl chain length specificity of Rhizopus delemar lipase through mutagenesis and molecular modeling
AUTHOR: Klein R R (Reprint); King G; Moreau R A; Haas M J
CORPORATE SOURCE: USDA ARS, ERRC, 600 E MERMAID LANE, WYNDMOOR, PA 19038 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: LIPIDS, (FEB 1997) Vol. 32, No. 2, pp. 123-130.
ISSN: 0024-4201.
PUBLISHER: AMER OIL CHEMISTS SOC, 1608 BROADMOOR DRIVE, CHAMPAIGN, IL 61821-0489.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 27
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The acyl binding site of Rhizopus delemar prolipase and mature lipase was altered through site-directed mutagenesis to improve lipase specificity for short- or medium-chain length fatty acids. Computer-generated structural models of R. delemar lipase were used in mutant protein design and in the interpretation of the catalytic properties of the resulting recombinant enzymes. Molecular dynamics simulations of the double mutant, val209trp + phe112trp, predicted that the introduction of trp112 and trp209 in the acyl binding groove would sterically hinder the docking of fatty acids longer than butyric acid. Assayed against a mixture of triacylglycerol substrates, the val209trp + phe112trp mature lipase mutant showed an 80-fold increase in the hydrolysis of tributyrin relative to the hydrolysis of tricaprylin while no triolein hydrolysis was detected. By comparison, the val94Trp mutant, predicted to pose steric or geometric constraints for docking fatty acids longer than caprylic acid in the acyl binding groove, resulted in a modest 1.4-fold increase in tricaprylin hydrolysis relative to the hydrolysis of tributyrin. Molecular models of the double mutant phe95asp + phe214arg indicated the creation of a salt bridge between asp95 and arg214 across the distal end of the acyl binding groove. When challenged with a mixture of triacylglycerols, the phe95asp + phe214arg substitutions resulted in an enzyme with 3-fold enhanced relative activity for tricaprylin compared to triolein, suggesting that structural determinants for medium-chain length specificity may reside in the distal end of the acyl binding groove. Attempts to introduce a salt bridge within 8 Angstrom of the active site by the double mutation leu146lys + ser1 15asp destroyed catalytic activity entirely. Similarly, the substitution of polar Gln at the rim of the acyl binding groove for phe112 largely eliminated catalytic activity of the lipase.

L50 ANSWER 23 OF 37 SCISEARCH COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1997:470292 SCISEARCH Full-text
THE GENUINE ARTICLE: XF115
TITLE: Computer-aided modelling of stereoselective triglyceride hydrolysis catalyzed by Rhizopus oryzae lipase
AUTHOR: Holzwarth H C (Reprint); Pleiss J; Schmid R D
CORPORATE SOURCE: UNIV STUTTGART, INST TECH BIOCHEM, D-70569 STUTTGART, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: JOURNAL OF MOLECULAR CATALYSIS B-ENZYMATIC, (10 JUN 1997) Vol. 3, No. 1-4, pp. 73-82. ISSN: 1381-1177.
PUBLISHER: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: English
REFERENCE COUNT: 28
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Lipase from Rhizopus oryzae catalyzes the stereoselective hydrolysis of triglycerides and analogues. Stereopreference and degree of enantiomeric excess of the product varies with the structure of the substrate: trioctanoylglycerol ('ester') and the sn-2 analogues 2-X-1,3-dioctanoylpropandiol, where 2-X = 2-O-octyl ('ether') and 2-hexyl('alkane'), are preferentially hydrolyzed at sn-1, substitution by 2-phenyl ('phenyl') reverses the stereopreference to sn-3. We have modelled the stereoselectivity of Rhizopus oryzae lipase by docking the tetrahedral intermediates of these substrates in two orientations, appropriate to hydrolysis at sn-1 or sn-3, respectively. The initial complexes were further relaxed by molecular dynamics simulations. The favoured orientation of a substrate is characterized by three factors: (1) The substrate fits well into the binding site. The glycerol backbone is relaxed and the sn-2 chain points to a well defined hydrophobic binding site. (2) The oxyanion is stabilized by an extra hydrogen bond from the side chain of Thr 83. (3) The substrate lacks repulsive interactions with protein side chains, especially of Leu 258.
Our model is consistent with experimental data and explains qualitatively the ranking of four different substrates with regard to stereoselectivity. It can be used to design lipase mutants with modified stereoselectivity.

L50 ANSWER 24 OF 37 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 97042947 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 8888145
TITLE: The consequences of engineering an extra disulfide bond in the Penicillium camembertii mono- and diglyceride specific lipase.
AUTHOR: Yamaguchi S; Takeuchi K; Mase T; Oikawa K; McMullen T; Derewenda U; McElhaney R N; Kay C M; Derewenda Z S
CORPORATE SOURCE: Tsukuba Research Laboratories, Amano Pharmaceutical Co. Ltd., Japan.

SOURCE: Protein engineering, (1996 Sep) Vol. 9, No. 9, pp. 789-95.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 14 May 1997
Last Updated on STN: 14 May 1997
Entered Medline: 6 May 1997

AB The extracellular lipase from Penicillium camembertii has unique substrate specificity restricted to mono- and diglycerides. The enzyme is a member of a homologous family of lipases from filamentous fungi. Four of these proteins, from the fungi Rhizomucor miehei, Humicola lanuginosa, Rhizopus delemar and P. camembertii, have had their structures elucidated by X-ray crystallography. In spite of pronounced sequence similarities the enzymes exhibit significant differences. For example, the thermostability of the P. camembertii lipase is considerably lower than that of the H. lanuginosa enzyme. Since only the P. camembertii enzyme lacks the characteristic long disulfide bridge, corresponding to Cys22-Cys268 in the H. lanuginosa lipase, we have engineered this disulfide into the former enzyme in the hope of obtaining a significantly more stable fold. The properties of the double mutant (Y22C and G269C) were assessed by a variety of biophysical techniques. The extra disulfide link was found to increase the melting temperature of the protein from 51 to 63 degrees C. However, no difference is observed under reducing conditions, indicating an intrinsic instability of the new disulfide. The optimal temperature for catalytic activity decreased by 10 degrees C and the optimum pH was shifted by 0.7 units to more acidic.

L50 ANSWER 25 OF 37 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 97015915 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 8862552
TITLE: The role of Glu87 and Trp89 in the lid of Humicola lanuginosa lipase.
AUTHOR: Martinelle M; Holmquist M; Clausen I G; Patkar S; Svendsen A; Hult K
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
SOURCE: Protein engineering, (1996 Jun) Vol. 9, No. 6, pp. 519-24.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 28 Jan 1997
Last Updated on STN: 29 Jan 1999
Entered Medline: 9 Dec 1996

AB The importance of Glu87 and Trp89 in the lid of Humicola lanuginosa lipase for the hydrolytic activity at the water/lipid interface was investigated by site-directed mutagenesis. It was found that the effect on the hydrolytic activity upon the replacement of Trp89 with Phe, Leu, Gly or Glu was substrate dependent. The Trp89 mutants displayed an altered chain length specificity towards triglycerides, with a higher relative activity towards triacetin and trioctanoin compared with tributyrin. Trp89 was shown to be less important in the hydrolysis of vinyl esters compared with ethyl esters and triglycerides. An exclusive effect on the acylation reaction rate by the mutation of Trp89 was consistent with the data. It is suggested that Trp89 is important in the process of binding the acyl chain of the substrate into the active site for optimal acylation reaction rate. The Trp89Phe mutation resulted in an increased hydrolytic activity towards 2-alkylalkanoic acid esters. This is suggested to be due to reduction of unfavourable van der Waals contacts between Trp89 and the 2-substituent of the substrate. Thus, in contrast to natural substrates, Trp89 has a negative impact on the catalytic efficiency when substrates with bulky acyl chains are used. In contrast to the Trp89 mutations, the effect on the hydrolytic activity of the Glu87Ala mutation was almost substrate independent, 35-70% activity of wild-type lipase. A reduction of both the acylation and deacylation reaction was consistent with the data.

L50 ANSWER 26 OF 37 LIFESCI COPYRIGHT 2007 CSA on STN
ACCESSION NUMBER: 1998:29753 LIFESCI Full-text
TITLE: Acyl glycerol hydrolases: Inhibitors, interface and catalysis
AUTHOR: Cambillau, C.; Longhi, S.; Nicolas, A.; Martinez, C.
CORPORATE SOURCE: Architecture et Fonction des Macromolecules Biologiques, UPR 9039, CNRS, IFRI, 31 Chemin Joseph Aiguier, 13402 Marseille, Cedex 20, France
SOURCE: CURR. OPIN. STRUCT. BIOL., (19960800) vol. 6, no. 4, pp.

449-455.
ISSN: 0959-440X.
DOCUMENT TYPE: Journal
TREATMENT CODE: General Review
FILE SEGMENT: K
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The last five years have witnessed the solution of a large number of lipase structures, which has led, among other insights, to the structural interpretation of the interfacial activation phenomenon in terms of 'lid' opening. This interpretation has been extended this year to include phospholipase A2. Recent structural studies on lipases have provided data on the detailed mechanisms underlying the behaviour of lipases: how they bind to inhibitors or substrates, and what interactions occur between their hydrophobic face and hydrophobic molecules, for example. In addition, studies on cutinase point mutants have shed some light on the role of the oxyanion hole in lipolytic catalysis.

L50 ANSWER 27 OF 37 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1996:113975 BIOSIS Full-text
DOCUMENT NUMBER: PREV199698686110
TITLE: Contribution of cutinase serine 42 side chain to the stabilization of the oxyanion transition state.
AUTHOR(S): Nicolas, Anne; Egmond, Maarten; Verrips, C. Theo; De Vlieg, Jakob; Longhi, Sonia; Cambillau, Christian [Reprint author]; Martinez, Chrislaine
CORPORATE SOURCE: Lab. Cristallographic Cristallisation des Macromolecules Biol., URA1296-CNRS, IFR1, 31 Chemin J. Aiguier, 13402 Marseille cedex 09, France
SOURCE: Biochemistry, (1996) Vol. 35, No. 2, pp. 398-410.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Mar 1996
Last Updated on STN: 10 Jun 1997
AB Cutinase from the fungus Fusarium solani pisi is a lipolytic enzyme able to hydrolyze both aggregated and soluble substrates. It therefore provides a powerful toot for probing the mechanisms underlying lipid hydrolysis. Lipolytic enzymes have a catalytic machinery similar to those present in serine proteinases. It is characterized by the triad Ser, His, and Asp (Glu) residues, by an oxyanion binding site that stabilizes the transition state via hydrogen bonds with two main chain amide groups, and possibly by other determinants. It has been suggested on the basis of a covalently bond inhibitor that the cutinase oxyanion hole may consist not only of two main chain amide groups but also of the Ser42 O-gamma side chain. Among the esterases and the serine and the cysteine proteases, only Streptomyces scabies esterase, subtilisin, and papain, respectively, have a side chain residue which is involved in the oxyanion hole formation. The position of the cutinase Ser42 side chain is structurally conserved in Rhizomucor miehei lipase with Ser82 O-gamma, in Rhizopus delemar lipase with Thr83 O-gamma-1, and in Candida antartica B lipase with Thr40 O-gamma-1. To evaluate the increase in the tetrahedral intermediate stability provided by Ser42 O-gamma, we mutated Ser42 into Ala. Furthermore, since the proper orientation of Ser42 O-gamma is directed by Asn84, we mutated Asn84 into Ala, Leu, Asp, and Trp, respectively, to investigate the contribution of this indirect interaction to the stabilization of the oxyanion hole. The S42A mutation resulted in a drastic decrease in the activity (450-fold) without significantly perturbing the three-dimensional structure. The N84A and N84L mutations had milder kinetic effects and did not disrupt the structure of the active site, whereas the N84W and N84D mutations abolished the enzymatic activity due to drastic steric and electrostatic effects, respectively.

L50 ANSWER 28 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1996:404098 CAPLUS Full-text
DOCUMENT NUMBER: 125:133994
TITLE: A URA3-promoter deletion in a pYES vector increases the expression level of a fungal lipase in Saccharomyces cerevisiae
AUTHOR(S): Okkels, Jens Sigurd
CORPORATE SOURCE: Novo Nordisk, Copenhagen, DK-2100, Den.
SOURCE: Annals of the New York Academy of Sciences (1996), 782(Recombinant DNA Biotechnology III), 202-207
CODEN: ANYAA9; ISSN: 0077-8923
PUBLISHER: New York Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Expression of a lipase gene from Humicola lanuginosa in Saccharomyces cerevisiae is increased, allowing large-scale purification A simple deletion of the URA3 promoter from a S. cerevisiae expression plasmid was performed. The promoter-deleted plasmid has an increased expression level

of fungal lipase gene. The deletion probably causes a poor expression of the URA3 selection marker, probably resulting in a higher copy number per cell of the plasmid. This higher copy number can increase the transcript level per cell and there by the expression level. In the case of the fungal lipase gene, the expression level with defined inoculum is increased at least three-fold. A part of the 2-μm origin of the pYES type plasmid was also deleted by the URA3 promoter deletion without affecting transformation frequency. The URA3 promoter can easily be deleted from most pYES type plasmids, since most of these plasmids only contain the two PstI sites that remove the whole URA3 promoter.

L50 ANSWER 29 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN DUPLICATE 9
ACCESSION NUMBER: 1996:490978 CAPLUS Full-text
DOCUMENT NUMBER: 125:215670
TITLE: Lipases from Humicola lanuginosa adsorbed to hydrophobic surfaces - desorption and activity after addition of surfactants
AUTHOR(S): Wannerberger, Kristin; Arnebrant, Thomas
CORPORATE SOURCE: Department of Food Technology, University of Lund, Box 124, Lund, S-22100, Swed.
SOURCE: Colloids and Surfaces, B: Biointerfaces (1996), 7(3/4), 153-164
CODEN: CSBBEQ; ISSN: 0927-7765
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The adsorption of lipase to methylated silica and its subsequent removal upon sequential addition of surfactants were measured in situ by ellipsometry. In addition, the specific activity of adsorbed lipase without, and after, exposure to surfactants was determined Two lipases from Humicola lanuginosa were studied, the wild-type (WT) and a mutant which was modified to increase the hydrophobicity of the active-site region. The surfactants used were SDS, C12E5 and sodium dodecanoate and they were all studied in the concentration range ≤ their critical micelle concentration (cmc). In all expts. a non-desorbable fraction remained at the surface. This fraction was larger, however, for the WT compared to the mutant. The concentration, expressed as a fraction of the cmc, of surfactant inducing the most pronounced desorption was found to vary among the surfactants. Desorption occurred at increasing fractions of the cmc in the order C12E5, sodium dodecanoate and SDS. This was found for both the WT and the mutant. No significant effect of C12E5 or SDS on the specific activities could be observed while the exposure to sodium dodecanoate markedly increased the specific activity of both lipases.

L50 ANSWER 30 OF 37 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 95242058 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 7536956
TITLE: The importance of non-charged amino acids in antibody binding to Humicola lanuginosa lipase.
AUTHOR: Naver H; Lovborg U
CORPORATE SOURCE: Novo Nordisk Industrial Biotechnology, Copenhagen, Denmark.
SOURCE: Scandinavian journal of immunology, (1995 May) Vol. 41, No. 5, pp. 443-8.
Journal code: 0323767. ISSN: 0300-9475.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 5 Jun 1995
Last Updated on STN: 29 Jan 1999
Entered Medline: 25 May 1995
AB The antigenicity of 36 Humicola lanuginosa lipase (HL) variants, generated by site directed mutagenesis, was compared with that of the unchanged enzyme. Polyclonal antibodies raised against variant lipases were investigated and compared with the antibodies raised against the wild type lipase in an ELISA competition assay. The results showed that exchange of charged amino acids with polar residues in surface epitopes of HL, results in a tighter binding of the antibody to the epitope. Four amino acids (Trp at position 89, Asp at positions 96 and 254 and Phe at position 211) were found to be essential for antibody binding in each their epitope of the wild type enzyme.

L50 ANSWER 31 OF 37 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 95391076 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 7662109
TITLE: Probing a functional role of Glu87 and Trp89 in the lid of Humicola lanuginosa lipase through transesterification reactions in organic solvent.
AUTHOR: Holmquist M; Clausen I G; Patkar S; Svendsen A; Hult K

CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
SOURCE: Journal of protein chemistry, (1995 May) Vol. 14, No. 4, pp. 217-24.
Journal code: 8217321. ISSN: 0277-8033.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 20 Oct 1995
Last Updated on STN: 29 Jan 1999
Entered Medline: 10 Oct 1995

AB To reveal the functional role of Glu87 and Trp89 in the lid of Humicola lanuginosa lipase, site-directed mutagenesis at Glu87 and Trp89 was carried out. The catalytic performance of wild-type and mutated lipases was studied in transesterification reactions in cyclohexane at a controlled water activity. Two different acyl donors were used in the investigation: tributyrin, a natural substrate for a lipase, and vinyl butyrate, an activated ester suitable for fast and efficient lipase-catalyzed transformations in preparative organic synthesis. As acyl acceptor 1-heptanol was used. The Glu87Ala mutation decreased the Vmax,app value with tributyrin and vinyl butyrate by a factor of 1.5 and 2, respectively. The Km,app for tributyrin was not affected by the Glu87Ala mutation, but the Km,app for vinyl butyrate increased twofold compared to the wild-type lipase. Changing Trp89 into a Phe residue afforded an enzyme with a 2.7- and 2-fold decreased Vmax,app with the substrates tributyrin and vinyl butyrate, respectively, compared to the wild-type lipase. No significant effects on the Km,app values for tributyrin or vinyl butyrate were seen as a result of the Trp89Phe mutation. However, the introduction of a Glu residue at position 89 in the lid increased the Km,app for tributyrin and vinyl butyrate by a factor of > 5 and 2, respectively. The Trp89Glu mutated lipase could not be saturated with tributyrin within the experimental conditions (0-680 mM) studied here. With vinyl butyrate as a substrate the Vmax,app was only 6% of that obtained with wild-type enzyme.

L50 ANSWER 32 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1995:340822 CAPLUS Full-text
DOCUMENT NUMBER: 122:155213
TITLE: Lipase variants, their manufacture with recombinant cells, and their use in detergent compositions
INVENTOR(S): Svendsen, Allan; Patkar, Shamkant Anant; Gormsen, Erik; Clausen, Ib Groth
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 5
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9425577	A1	19941110	WO 1994-DK162	19940422
W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KG, KP, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, TJ, UA, US, UZ, VN				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9465358	A	19941121	AU 1994-65358	19940422
BR 9406384	A	19960116	BR 1994-6384	19940422
EP 695348	A1	19960207	EP 1994-913051	19940422
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, NL, PT, SE				
CN 1124039	A	19960605	CN 1994-192175	19940422
JP 08509364	T	19961008	JP 1994-523626	19940422
FI 9505018	A	19951020	FI 1995-5018	19951020
PRIORITY APPLN. INFO.:			DK 1993-466 A	19930423
			WO 1994-DK162 W	19940422

AB Novel lipase variants with improved properties, DNA constructs coding for the expression of these variants, host cells expressing the lipase variant genes, and methods for preparing the variants by culturing the recombinant cells are claimed. Lipases comprising a trypsin-like catalytic triad including an active serine located in a predominantly hydrophobic, elongated binding pocket of the lipase mol. are mutated so as to substitute a non-aromatic amino acid residue of a lipid contact zone comprising residues located within the part of the lipase structure containing the active serine residue, which residues may participate in the interaction with the substrate at or during hydrolysis, with an aromatic amino acid residue. Several Humicola lanuginosa lipase variants were produced with Aspergillus oryzae and tested for wash performance. The [Trp-96]-, and [Trp-96,Asn-210]- lipase variants exhibited 2.7 and 3.4-fold better wash performance than wild-type lipase.

L50 ANSWER 33 OF 37 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 95115502 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 7815893
TITLE: Trp89 in the lid of Humicola lanuginosa lipase is important for efficient hydrolysis of tributyrin.
AUTHOR: Holmquist M; Martinelle M; Clausen I G; Patkar S; Svendsen A; Hult K
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
SOURCE: Lipids, (1994 Sep) Vol. 29, No. 9, pp. 599-603.
Journal code: 0060450. ISSN: 0024-4201.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 17 Feb 1995
Last Updated on STN: 29 Jan 1999
Entered Medline: 9 Feb 1995

AB To determine whether Trp89 located in the lid of the lipase (EC 3.1.1.3) from Humicola lanuginosa is important for the catalytic property of the enzyme, site-directed mutagenesis at Trp89 was carried out. The kinetic properties of wild type and mutated enzymes were studied with tributyrin as substrate. Lipase variants in which Trp89 was changed to Phe, Leu, Gly or Glu all showed less than 14% of the activity compared to that of the wild type lipase. The Trp89Glu mutant was the least active with only 1% of the activity seen with the wild type enzyme. All Trp mutants had the same binding affinity to the tributyrin substrate interface as did the wild type enzyme. Wild type lipase showed saturation kinetics against tributyrin when activities were measured with mixed emulsions containing different proportions of tributyrin and the nonionic alkyl polyoxyethylene ether surfactant, Triton DF-16. Wild type enzyme showed a Vmax = 6000 +/- 300 mmol.min-1.g-1 and an apparent Km = 16 +/- 2% (vol/vol) for tributyrin in Triton DF-16, while the mutants did not show saturation kinetics in an identical assay. The apparent Km for tributyrin in Triton DF-16 was increased as the result of replacing Trp89 with other residues (Phe, Leu, Gly or Glu). The activities of all mutants were more sensitive to the presence of Triton DF-16 in the tributyrin substrate than was wild type lipase. The activity of the Trp89Glu mutant was decreased to 50% in the presence of 2 vol% Triton DF-16 compared to the activity seen with pure tributyrin as substrate.(ABSTRACT TRUNCATED AT 250 WORDS)

L50 ANSWER 34 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1995:18786 CAPLUS Full-text
DOCUMENT NUMBER: 122:34002
TITLE: Detergent compositions comprising lipase variants
AUTHOR(S): Novo, Nordisk
CORPORATE SOURCE: Den.
SOURCE: Research Disclosure (1994), 359, 151-6 (No. 35944)
CODEN: RSDSBB; ISSN: 0374-4353
DOCUMENT TYPE: Journal; Patent
LANGUAGE: English
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
RD 359044		19940310		

PRIORITY APPLN. INFO.: RD 1994-359044 19940310

AB Detergent compns., especially for dishwashing, contain lipase from Humicola lanuginosa for improved effectiveness.

L50 ANSWER 35 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1993:576669 CAPLUS Full-text
DOCUMENT NUMBER: 119:176669
TITLE: Improving the resistance of proteins and enzymes to proteolysis by manipulation of surface structures
INVENTOR(S): Svendsen, Allan; Clausen, Ib Groth; Pathar, Shamkant Anant
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 55 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9311254	A1	19930610	WO 1992-DK351	19921126
W: BR, CA, FI, JP, KR, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
JP 07504807	T	19950601	JP 1992-509724	19921126
EP 667915	A1	19950823	EP 1992-924587	19921126
R: AT, BE, DE, DK, ES, FR, GB, IT, NL				
BR 9206815	A	19951031	BR 1992-6815	19921126
FI 9402467	A	19940526	FI 1994-2467	19940526
PRIORITY APPLN. INFO.:			WO 1991-DK350 A	19911126
			DK 1950-91003 A	19911126
			DK 1950-92003 A	19911126
			WO 1992-DK351 W	19921126

AB Enzymes or other proteins are made more resistant to proteinases by modification of the sequences of surface loops that are common sites of proteolysis. The method is particularly suited for enzymes to be used in proteinase-containing laundry detergents. These sites are identified by very limited proteolysis of the protein and N-terminal sequencing of the cleavage products. Three cleavage sites identified the lipase of Humicola lanuginosa DSM 4106 at positions 162-165, 209-212, and 239-242 were substituted with an array of tetrapeptides by standard methods of site-directed mutagenesis of the cloned gene and the proteins manufactured by expression of the gene in Aspergillus oryzae. Under conditions in which the wild-type proteinase lost 75% of its activity in 90 min upon digestion with Savinase® (subtilisin), one of these variants retained >80% of its activity.

L50 ANSWER 36 OF 37 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 94183411 MEDLINE Full-text
DOCUMENT NUMBER: PubMed ID: 8136025
TITLE: Lipases from Rhizomucor miehei and Humicola lanuginosa: modification of the lid covering the active site alters enantioselectivity.
AUTHOR: Holmquist M; Martinelle M; Berglund P; Clausen I G; Patkar S; Svendsen A; Hult K
CORPORATE SOURCE: Department of Biochemistry and Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
SOURCE: Journal of protein chemistry, (1993 Dec) Vol. 12, No. 6, pp. 749-57.
Journal code: 8217321. ISSN: 0277-8033.
PUB. COUNTRY: United States
DOCUMENT TYPE: (COMPARATIVE STUDY)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 9 May 1994
Last Updated on STN: 29 Jan 1999
Entered Medline: 28 Apr 1994

AB The homologous lipases from Rhizomucor miehei and Humicola lanuginosa showed approximately the same enantioselectivity when 2-methyldecanoic acid esters were used as substrates. Both lipases preferentially hydrolyzed the S-enantiomer of 1-heptyl 2-methyldecanoate (R. miehei: ES = 8.5; H. lanuginosa: ES = 10.5), but the R-enantiomer of phenyl 2-methyldecanoate (ER = 2.9). Chemical arginine specific modification of the R. miehei lipase with 1,2-cyclohexanedione resulted in a decreased enantioselectivity (ER = 2.0), only when the phenyl ester was used as a substrate. In contrast, treatment with phenylglyoxal showed a decreased enantioselectivity (ES = 2.5) only when the heptyl ester was used as a substrate. The presence of guanidine, an arginine side chain analog, decreased the enantioselectivity with the heptyl ester (ES = 1.9) and increased the enantioselectivity with the aromatic ester (ER = 4.4) as substrates. The mutation, Glu 87 Ala, in the lid of the H. lanuginosa lipase, which might decrease the electrostatic stabilization of the open-lid conformation of the lipase, resulted in 47% activity compared to the native lipase, in a tributyrin assay. The Glu 87 Ala mutant showed an increased enantioselectivity with the heptyl ester (ES = 17.4) and a decreased enantioselectivity with the phenyl ester (ER = 2.5) as substrates, compared to native lipase. The enantioselectivities of both lipases in the esterification of 2-methyldecanoic acid with 1-heptanol were unaffected by the lid modifications.

L50 ANSWER 37 OF 37 CAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1993:142540 CAPLUS Full-text
DOCUMENT NUMBER: 118:142540
TITLE: Stabilization of enzymes by selective replacement of amino acids with proline

INVENTOR(S): Svendsen, Allan; Von der Osten, Claus; Clausen, Ib Groth; Patkar, Shamkant Anant; Borch, Kim
PATENT ASSIGNEE(S): Novo Nordisk A/S, Den.
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9219726	A1	19921112	WO 1992-DK142		19920501
W: FI, JP, KR, US					
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE					
EP 585285	A1	19940309	EP 1992-910123		19920501
EP 585285	B1	19980812			
R: AT, BE, DE, DK, ES, FR, GB, IT, NL, SE					
JP 06507071	T	19940811	JP 1992-509154		19920501
AT 169678	T	19980815	AT 1992-910123		19920501
ES 2121854	T3	19981216	ES 1992-910123		19920501
US 5914306	A	19990622	US 1993-140008		19931022
PRIORITY APPLN. INFO.:			EP 1991-610035	A	19910501
			WO 1992-DK142	W	19920501

AB Enzymes are made more stable by methods that minimize the structural flexibility of the peptide backboné. This is achieved by substituting selected amino acids with proline in regions of the protein outside stable higher order structures such as α-helices and β-sheets. The criteria for selecting amino acids to be substituted also include dihedral angles ψ and ϕ in the ranges -90°<ϕ<-40° and -180°<ψ<-150°, -80<ψ<10, 100°<ψ<180. The invention is intended for use in the preparation of stable enzymes for use in detergents. Genes for a set of derivs. of the lipase of Humicola lanuginosa with individual amino acids substituted by proline weré prepared by PCR mutagenesis of the gene and the expression cassette used to manufacture the protein in Aspergillus. An analog of the lipase with Gly225 replaced by Pro lost 50% of its activity after .apprx.30 min at 70° whereas the wild type lost 50% of its activity after 15 min. at the same temperature

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